

THE MECHANISMS BEHIND ALTERNATIVE COMMUNITY STATES:
UNDERSTANDING PRODUCER COMPOSITION IN TEMPORARY AND
SEMIPERMANENT WETLANDS

BY

SIGRID SMITH

DISSERTATION

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Doctoral Committee:

Associate Professor Carla Cáceres, Chair
Professor Daniel Schneider
Associate Professor Andrew Suarez
Assistant Professor Spencer Hall, Indiana University

ABSTRACT

In many ecological communities, variation in composition appears discontinuous, in that a discrete set of community states (or distinct taxa assemblages) have been observed repeatedly in one ecosystem type. Many hypotheses have been proposed to explain this discontinuous variation in community composition over space and time. I examined the applicability of these hypotheses to understand the structure of producer communities of temporary and semipermanent wetlands in Michigan, USA. I identified several distinct community states, with each characterized by dominance of particular functional groups (submerged, floating, or emergent plants) or the absence of plants throughout a season.

I developed a framework for differentiating among these hypotheses by characterizing how community composition varies (1) over space, (2) over time, and (3) with possible drivers. I applied this framework to my survey data of the producer communities of wetlands over four years ($n = 21\text{-}35$ wetlands). I found that several environmental variables (pH, light, depth, and permanence) were associated with community composition based on multivariate analyses in these wetlands. Alternative stable states or interactions among several drivers were the most strongly supported explanations for the expression of multiple community states.

I further examined factors driving dominance of free-floating plants in wetland producer communities in two additional studies. I evaluated the roles of nitrogen (N), phosphorus (P), and N:P ratio in driving floating plant dominance in a separate survey dataset and in a field experiment. In the observational work, N, P, and N:P ratios all were associated with producer community composition. I manipulated the three variables in enclosures in two experimental ponds with floating and submerged plants. I found N supply rates drove these patterns more

strongly than P and N:P. However, submerged plant composition and pH levels also affected floating plant responses.

Finally, I evaluated the role of light in changing the response of floating plants to N. In my observational data, I found that submerged and emergent plants only dominated wetlands with open tree canopies, and that floating plants more consistently dominated systems at intermediate N levels when light availability was low. To investigate this relationship further, I manipulated light, N, and initial densities of floating plants in an outdoor mesocosm experiment with floating plants and algae. In this experiment, I found that floating plants exhibited a light x nutrient interaction. Competition with other plant groups and photoinhibition are discussed as possible mechanisms driving these patterns.

In summary, a complex suite of environmental variables drives the expression of multiple community states in temporary and semipermanent wetlands. While there is some support for the hypothesis that nutrients and light drive alternative stable states between floating plants and other producers, several other variables play important roles in regulating dominance of floating plants as well.

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CHAPTER 1

INTRODUCTION: POSSIBLE CAUSES OF DISCONTINUOUS VARIATION IN NATURAL COMMUNITIES

Introduction:

Ecologists have sought to understand the membership and abundances of species in communities for centuries. Based on successional theory, ecologists often have expected communities to remain stable over relatively long periods of time. Thus, abrupt changes in community structure over time and space have been perplexing. Individual populations increase and crash within communities, with some populations rarely remaining at intermediate levels (e.g., larch budmoths, lemmings) (May 1977, Turchin 2003). Groups of species overturn with time as well (e.g., sand dune vegetation: Cowles 1899 and Miller et al. 2010, transitions between grasslands and woodlands: Dublin et al. 1990, collapses of ocean fisheries: Jackson et al. 2001). Over space, community structure can appear patchy, even in similar environments (e.g., treefall gaps in forests: Lin et al. 2004; necrophagous insect communities in carrion: Beaver 1977). These stark differences in community structure over space and time are important to understand not only to deepen our basic knowledge of ecosystem processes but also to improve ecosystem management, since many of the above changes represent major management challenges (e.g., insect outbreaks, fisheries collapsing).

However, the causes of discontinuous variation in natural communities are not always clear. Many hypotheses and theories have been proposed (see next section, Table 1.1). Several of these theories, particularly succession (Clements 1936, Gleason 1926, Kingsland 1991, Pickett et al. 2009) and alternative stable states theory (e.g., Connell and Sousa 1983 and responses, Carpenter 2001,

Schröder et al. 2005), have been hotly debated. This dizzying array of possibilities can be confusing to sort through when tackling a new system. Ecosystem complexity adds yet another layer of complication--several drivers and their interactions may be involved in generating and shifting the drivers of discontinuous variation, and several time and spatial scales may be involved (e.g., interactions of fast and slow variables to generate bifurcations: Rinaldi and Scheffer 2000, Groffman et al. 2006). This chapter presents an attempt to organize possible causes of discontinuous variation to evaluate their support in understudied systems.

Hypotheses, theories, and paradigms abound:

Many hypotheses have been invoked individually to explain discontinuous variation in natural communities (Table 1.1). These hypotheses range over several scales of biological organization, from individual populations to whole ecosystems. The drivers of community variation may be biotic or abiotic (or a combination of the two) and may act on different time scales. These hypotheses are not mutually exclusive; on the contrary, many of the hypotheses and theories discussed later are built on earlier discussed mechanisms.

(Table 1.1.1) Disturbance can lead to discontinuous variation in community structure, especially in concert with biological mechanisms. Disturbances (e.g., fire or insect outbreaks in a forest, wave action in an intertidal zone) may create heterogeneity in a contiguous habitat (e.g., Lin et al. 2004). On a local scale, these disturbances may create small patches within a larger system. These patches may represent transient variation if they are immediately colonized by the same suite of species constituting the rest of the community, or they may generate persistent variation if the patches maintain these differences or diverge further in structure (Connell 1978). While differences in species colonization

may result from pure chance, differences may also result from dispersal limitation (Morin 1999). Dispersal limitation may create variation in community structure unto itself as well, especially in discrete habitat patches (MacArthur and Wilson 1967, Holyoak et al. 2005).

(Table 1.1.2) Small differences in initial community structure can lead to largely different communities from diverging trajectories during community assembly (Hastings et al. 1993). Priority effects can scale up small stochastic differences in colonization into large differences in community structure. This is the basis of chaos theory, which has been discussed in many fields beyond ecology (Hastings et al. 1993).

(Table 1.1.3) Internal population cycles may also lead to differences in the abundance of one population, which can have cascading effects on other species. Some species have long development times (e.g., 13 or 17 years in periodic cicadas *Magicicada*: Gullan and Cranston 2000, Clay et al. 2009), leading to different community level responses when adults vs. immature individuals are abundant (Clay et al. 2009). In addition, transient oscillations within a population are possible. For example, modeling and laboratory studies show that individual populations with sigmoidal growth responses can overshoot their carrying capacity and then exhibit dampening cycles as the population size approaches carrying capacity (Morin 1999).

(Table 1.1.4-5) Species interactions play foundational roles in community ecology, and they can cause discrete variation in community structure over space or time in several ways (Morin 1999). Interference or exploitative competition can lead to monocultures of a competitor that cannot be invaded (e.g., barnacles: Connell 1961). In addition, predator-prey interactions can lead to oscillatory cycles with booms and busts of individual species (e.g., lynx-hare cycles: Begon et al. 2006).

(Table 1.1.6) Many ideas here deal with discontinuous responses of organisms to continuous drivers (e.g., gradients of resources) in their environment, but the resource itself may be discontinuously distributed instead (Carpenter 2001). In some systems, discrete habitat patches are obvious (e.g., ponds in a terrestrial landscape), but they can be less obvious if the variable characterizing those differences has not been identified and measured. For example, plant communities may have a discrete change in structure with different geologic formations in grasslands (e.g., Harrison et al. 2006), and waters may have different oxygen levels with depth in a stratified lake (Wetzel 2001).

(Table 1.1.7) Populations and communities also may respond discontinuously to a continuous driver with thresholds (Groffman et al. 2006). One possibility is a simple threshold response to a driver (as in a step function in mathematics). For example, many aquatic species have physiological tolerances for specific ranges of water chemistry parameters beyond which they cannot persist, leading to a discontinuous pattern when surveying populations across several sites (e.g., caddisflies with oxygen and temperature: Colburn 2004, zooplankton with salinity: Wetzel 2001). More complicated nonlinear community responses, such as a hysteresis (S-shaped response curve in alternative stable states theory) may generate distinct community states despite a smooth change in a driver as well. A hysteresis may result in cases where a slowly changing driver affects fast-changing ecosystem variables differently at different values--in other words, an interactive effect occurs, such that several equilibrium states are possible (e.g., Rinaldi and Scheffer 2000). Empirically, such a response can be generated simply from several of the mechanisms already discussed. For example, two keystone species (representing different community types) may compete assymmetrically for a few limiting resources, and priority effects, resource sequestration, or other habitat alterations may serve as reinforcing feedback mechanisms once a given community has developed (Scheffer et al. 1993, Scheffer 2004).

(Table 1.1.8) Succession is another major theory in ecology that tries to explain strong differences in community composition over space and time (Begon et al. 2006, Pickett et al. 2009). An interplay of many mechanisms (e.g., disturbance, dispersal limitation, priority effects, competition) leads to directional, predictable change in species composition over time (Pickett et al. 1987, 2009). This occurs due to competition-colonization tradeoff, in which early in community assembly species with high dispersal ability will colonize and dominate a new habitat, but later the community will be dominated by stronger competitors. Many formulations and variants have been proposed, such as cyclic succession and polyclimax theory as well (e.g., Pickett et al. 1987).

(Table 1.1.9) Transient dynamics have been discussed as an alternative hypothesis for the former ideas as well (e.g., Connell 1978, Connell and Sousa 1983). Communities may be far from equilibrium due to frequent disturbance or tending toward a moving attractor (disequilibrium dynamics), or an equilibrium may not be possible due to a lack of species interactions stabilizing a community (nonequilibrium dynamics) (Connell 1978, Ellis and Swift 1988, Illius and O'Connor 1999).

More ideas building on and modifying expression of these mechanisms:

In addition to alternative stable states and succession, other combinations of these mechanisms have been proposed to generate discontinuous patterns in community structure. Metacommunity theory incorporates many of these mechanisms (e.g., dispersal limitation, competition) along with adaptation to parse out the causes of differences in community structure with discrete habitat patches (Holyoak et al. 2005). State-and-transition models, commonly used in rangeland ecology, incorporate both alternative stable states and succession theory to understand ecosystem dynamics (Westoby et al.

1989). Finally, panarchy theory (adaptive cycles) scales up mechanisms leading to alternative stable states--the interaction of variables changing at different speeds--into a broader theory incorporating disturbance, succession, and thresholds (Holling 1987).

Other mechanisms may interact with these mechanisms as well, changing the level of organization at which discontinuous variation is observed. For example, even mechanisms listed as affecting a single population can have ecosystem-level structural consequences. Populations can be strongly coupled via species interactions, particularly when a keystone species affects other biotic and abiotic components of their ecosystem (Paine 1969, Morin 1999). In addition, trophic cascades can link populations across a large community through predator-prey interactions (Carpenter and Kitchell 1993). Finally, ecosystem engineers can change the abiotic structure of an environment, leading to effects on other species (Jones et al. 1994).

Interactions between drivers and mechanisms can occur in other ways. In individual systems, more singular interactions may occur. For example, Harrison (1997) describes community differences based on dispersal limitation of a predator (tussock moth) coupled with predator-prey (grazing) cycles. Similarly, Sim and colleagues (2006, 2009) have described multiple different drivers (e.g., salinity, water regime, nutrients) interacting to create multiple distinct community states in Australian salinized wetlands. More generally, slow secondary drivers may lead to shifts in the effects of primary drivers in ecosystems as well (Groffman et al. 2006). For example, in a system with alternative stable states, variation in a secondary driver may change the shape of a hysteretic response curve to a main control factor, such that alternative stable states can only exist in certain conditions (e.g., Genkai-Kato and Carpenter 2005).

Conclusion:

Humans are accelerating changes in slow drivers such as climate (global climate change), nutrient cycling (eutrophication in lakes and ponds), and disturbance (fire regime changes in rangelands) (Chapin et al. 2006). With these changes, communities have been predicted to exhibit novel structures and complex behaviors, making discontinuous variation more likely in natural ecosystems (Groffman et al. 2006, Fox 2007, Seastedt et al. 2008). While many ideas have been proposed to explain such variation, empirical work diagnosing which mechanisms are acting in individual systems will be necessary to gain a full understanding of their dynamics. In this volume, I aim to understand expression of multiple community states in the producer communities of temporary and semipermanent wetlands in Michigan, USA. I both propose a framework to sort amongst these hypotheses using my observational data (Chapter 2) and use experimental data (Chapters 3 and 4) to understand how individual drivers may scale up to create the complex dynamics I have observed in these systems.

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Table:

Table 1.1. Hypotheses and theories for discontinuous variation in natural communities. Ideas proposed to explain instances in which starkly different communities have been observed over space or time. I include whether the hypothesis is an explicit, mechanistic process ("mechanism") or potentially includes several mechanisms ("emergent"), the level of organization at which each generates patterns, how a system varies over space (i.e. whether the hypothesis explicitly will generate patchy patterns), how a system varies over time (i.e. whether the hypothesis explicitly will generate particular patterns over time), and whether particular responses to drivers or feedback mechanisms are involved.

Hypothesis or theory	Mechanism	Level of organization	Variation over space	Variation over time	Driver (shape) and/or feedback
1. Stochastic extinction and colonization (inc. dispersal limitation) events, especially in the context of patch-based theories (e.g., metacommunities)	Mechanism	All scales	Yes	n/a	n/a
2. Chaos, priority effects	Emergent	Population or community	n/a	Divergence	Feedbacks
3. Internal population cycles (e.g., life cycle/development time)	Mechanism	Population	n/a	Pattern (cyclic)	n/a
4. Competition	Mechanism	Community	n/a	Persistence	Driver(s)
5. Species interactions tightly linking species in oscillatory cycles (e.g., predator-prey cycles)	Mechanism	Community	n/a	Pattern (cyclic)	Feedbacks

Table 1.1 (cont.)

6. Patchy habitat: 1+ species respond continuously to an environmental variable that is distributed discontinuously	Mechanism	Population or community	Yes	Persistence	Discontinuous driver (linear response)
7. Discontinuous response of 1+ species to continuous change in environment (thresholds, bifurcations)					
**7a. Simple threshold response	Mechanism	Population or community	n/a	Persistence	Driver (threshold response)
**7b. Alternative stable states (hysteresis)	Emergent	Community or ecosystem	n/a	Persistence, except with large disturbances	Driver (hysteretic response); feedbacks
8. Succession (and variations on it, like cyclic succession and polyclimax theory)	Emergent	Community	n/a	Pattern (directional*)	Feedbacks (and time)
9. Transient dynamics (nonequilibrium and disequilibrium)	n/a	All scales	n/a	No	n/a

*but see Pickett et al. (2009): current broader views do not require directional change toward a climax

CHAPTER 2

A FRAMEWORK FOR IDENTIFYING POSSIBLE CAUSES OF DISCONTINUOUS VARIATION IN COMMUNITY STRUCTURE

Abstract:

Many hypotheses have been proposed to explain the discontinuous variation often observed in community composition over space and time. I suggest a framework for differentiating among these possibilities by characterizing (1) how community composition varies over space, (2) how community composition varies over time, and (3) the possible drivers of this variation. I applied this approach to the producer communities of temporary and semipermanent wetlands in Michigan, USA. I identified several distinct community states, each characterized by dominance of particular functional groups (submerged, floating, or emergent plants) or the absence of plants throughout a season. These community states were not clustered together spatially, suggesting that dispersal limitation does not explain these patterns. Communities often shifted between states among years, but they rarely shifted within a season. Moreover, these temporal shifts were not ordered in a way to suggest directional or cyclic changes. Several environmental variables (e.g., pH, light, depth) had nonlinear associations with community composition. Interactions among several drivers best explained the expression of alternative community states. Formalizing initial data collection within this framework may help us understand less studied and rapidly changing ecosystems.

Introduction:

Ecologists often observe abrupt changes in community composition over space and time.

In many systems this variation in community composition appears discontinuous, in that a discrete set of community states (or distinct species assemblages) are observed repeatedly in one ecosystem type. Diverse examples of this discontinuous variation are seen in community structure over time, from rotifers and algae oscillating in small laboratory microcosms (Yoshida et al. 2003) to whole ecosystems precipitously collapsing in oceans and deserts in nature (Jackson et al. 2001, Scheffer et al. 2001, Scheffer and Carpenter 2003). Discrete variation in community composition is seen over space as well, including the dominance of different producers in shallow lakes (e.g., phytoplankton versus submerged plants: Scheffer et al. 2001) and in semiarid rangelands (e.g., grasses versus shrubs: Westoby et al. 1989). A variety of explanations have been proposed for this variation, including population cycles, spatial variation in resources, nonlinear responses of species to their environment, alternative stable states, and succession (Morin 1999, Scheffer and Carpenter 2003, Turchin 2003). However, it is difficult to determine which mechanisms are most important in natural systems, and the criteria required to do so have been controversial (Connell and Sousa 1983, Peterson 1984, Sousa and Connell 1985, Sutherland 1990, Price and Morin 2004).

While many studies have evaluated individual hypotheses to explain stark differences in community composition, no single hypothesis has proven robust across systems. In some systems, environmental gradients may drive variation in community composition. For example, environments may be patchy at large or small scales, and taxa may have gradual responses to discontinuously distributed drivers (e.g., endemic plant communities on harsh serpentine soils: Harrison et al. 2006). Alternately, apparent variation may represent infrequent sampling of transient dynamics (Connell and Sousa 1983). Theories such as alternative stable states (multiple community states reinforced by positive feedbacks, leading to an S-shaped response of

the community to a driver) and succession (predictable community changes over time with distinct stages) have been developed to explain discontinuous variation in community composition as well. The insight gained by blending succession and alternative stable states into state and transition models (Westoby et al. 1989, Zweig and Kitchens 2009) highlights the need to understand the full range of hypotheses for discontinuous variation, how their predictions differ, and how they may interact.

The aquatic plant communities of temperate wetlands are one system for which multiple hypotheses have been proposed to explain discontinuous variation in community structure. For example, free-floating duckweeds (Lemnaceae) and some submerged species, such as Eurasian water milfoil (*Myriophyllum spicatum*), are known to occur in high abundances in some systems but to be absent from others (Hillman 1961, Chambers et al. 1993). Changes in the abundances of emergent vegetation over time (e.g., cattails *Typha* sp.) are equally well documented (Caitlin and Hayden 1927 as cited in van der Valk 2005). These patterns may be driven by several mechanisms. Dispersal may limit formation of new populations and intolerance to high pH may cause extinction of extant populations of duckweeds *Lemna minor* and *L. triscula* (Keddy 1976). Physiological tolerance to particular water depths for germination and survival of emergent vegetation also has been proposed, which may generate cyclic patterns in community structure with cyclic changes in hydrologic regime (van der Valk 2005). At the community level, succession after disturbance (McMaster and McMaster 2001, Ray et al. 2001, Stroh et al. 2008) and alternative stable states (e.g., floating versus submerged plant dominance: Scheffer et al. 2003, Scheffer 2009) have been discussed to explain community structure in these systems as well. Also, transient dynamics typical of high disturbance systems (Connell and Sousa 1983) may be relevant, due to the periodic drying of temporary wetlands.

In this chapter, I first develop a novel framework aimed at disentangling multiple possible causes of discontinuous variation. In this conceptual model, I focus on the spatial and temporal variability in community composition and how this variation is associated with possible drivers (Figure 2.1). Second, I applied this model to understand variation in the composition of producer communities in temporary and semipermanent wetlands in southwest Michigan, USA. I used observational data to evaluate a broad set of hypotheses for discontinuous variation in these wetlands.

Methods:

Conceptual model:

In this model, I aimed to disentangle several hypotheses for discontinuous variation in community structure. I first evaluated whether discontinuous variation in producer community composition was present. I then differentiated several hypotheses that could explain these patterns by considering predictions that can be generated from each of them, as described below (Figure 2.1). To accomplish this, I focused on how community composition relates to geographic distance between sites, how composition changes over time, and how communities respond to possible drivers (Figure 2.1). In applying this framework, I used multivariate statistical techniques for most analyses, because they allowed an integrated view of community structure.

Characterizing composition (Figure 2.1.1)

In this framework, I first aimed to identify whether there is discontinuous variation expressed in the communities of interest. To do this, I characterized community composition at the level of both individual taxa and the community as a whole. Univariate techniques (e.g., tests

of bimodality) and multivariate tests (e.g., cluster analysis) can be used to evaluate community composition for discontinuous variation at both levels of organization.

Spatial variation (Figure 2.1.2)

Some hypotheses may be distinguishable based on the spatial patterns they generate. For example, dispersal limitation (which could generate distinct assemblages from species only colonizing some areas) and associations of taxa with patchily distributed habitats both would generate distinct patterns of spatial clustering when examining community composition over space. Models that detect spatial autocorrelation could be used to detect these patterns (Legendre and Legendre 1998). These two hypotheses can be distinguished further by examining associations with environmental variables (Tuomisto et al. 2003) or by fitting observed patterns of abundance to predictions made by diffusion models for species where dispersal is discrete and spatially restricted (Case 2000).

Temporal variation (Figure 2.1.3)

Monitoring communities over multiple time points allows for further evaluation of hypotheses creating discontinuous variation in community composition. The amount of change in an unmanipulated system, referred to with concepts like constancy and persistence, is particularly relevant (Grimm and Wissel 1997). Beyond helping to identify cyclic dynamics at the population level, stability plays a pivotal role in community-level theories. For example, directional change is a key feature of succession, as is the stability in alternative 'stable' states. Characterizing temporal patterns within and across communities for signatures of cyclic changes, directional changes, and frequent persistence may distinguish amongst these hypotheses. When lacking evidence for persistence or predictable change, transient dynamics (e.g., from nonequilibrium (no equilibrium possible) and disequilibrium (movement toward an equilibrium)

paradigms: Ellis and Swift 1988, Illius and O'Connor 1999) may be useful to consider.

Drivers (Figure 2.1.4)

Identifying possible drivers of community variation can give additional information to evaluate hypotheses, particularly to explain persistent discontinuous patterns in species composition. Environmental factors that represent limiting resources or abiotic constraints often can be identified at the outset of studies based on previous work. Biological drivers, such as grazing pressure or predator abundances, may be identified for study as well. Characterizing how community composition relates to these possible drivers can be useful, particularly by describing the shapes of taxa responses to these factors. For example, alternative stable states theory specifically implies a hysteresis ('S'-shaped response curve) to a driver, while a simpler threshold response may indicate simple physiological tolerances along an environmental gradient. This can be accomplished by evaluating the relative support for several models of possible species responses or by identifying thresholds in responses (De'ath and Fabricius 2000, Anderson 2008). When multiple possible drivers are identified a priori, multivariate techniques can be used to assess their relative importance (Legendre and Legendre 1998), and comparisons among techniques can help to elucidate the shapes of responses (see below).

Study system:

To examine the utility of the conceptual model, I collected data on temporary and semipermanent depressional wetlands in Barry, Kalamazoo, and Calhoun Counties, Michigan, USA. All wetlands were <1.3 m deep when initially sampled so that I could sample in waders. They were predominantly fishless. Much of my community characterization focused on functional groups, classifying plants by their placement in the water column (similar to

Sculthorpe 1967). I defined submerged plants as those rooted in the sediment with leaves not extending above the water surface. Emergent plants were rooted in the sediment with leaves frequently extending above the surface. Although pond lilies (*Nuphar* sp.) are less strongly rooted with rhizomes above the sediment, they were classified in this group, since most of their biomass is above the surface and they reach the bottom. Finally, floating plants were defined as plants with all parts at or near the surface of the water. While periphyton sometimes reached high abundances at the beginning of the growing season, it was uncommon (seen in only four of 26 wetlands in April 2006) and did not seem to play a major role in communities throughout the season. Phytoplankton more commonly reached high abundances, so these producers were included.

Survey methods and analysis:

Characterizing composition

To characterize community composition and its variation over space, I surveyed plant community composition and phytoplankton abundance in late summer 2006 (17 July-5 August; n=35 wetlands). As a proxy of phytoplankton abundance, water was collected for chlorophyll *a* content. I filtered 25-100 ml of water onto GF/F glass microfiber filters (Whatman, Maidstone, England); chlorophyll was extracted in cold 95% ethanol for approximately 24 hours and measured with narrow band fluorometry (Welschmeyer 1994).

I estimated floating and emergent plant cover as a percentage of the water surface by visual observation, and I estimated submerged plant cover within five 10x10 cm sinking quadrats (or three quadrats when submerged plants were extremely abundant). For the submerged plants, quadrats were haphazardly placed along a transect across each wetland, excluding edge

vegetation (cattail, *Typha* sp.) when present. I used my hands and/or a four-tine cultivator to bring the plants to the surface, and cover of each genus was estimated as the percentage of each quadrat occupied. For most analyses here, quadrats and genera were pooled for whole wetland cover estimates by functional group. The sum of all three functional groups sometimes exceeded 100 percent, since each functional group had biomass concentrated on different planes of wetlands (i.e. the wetland basin for submerged plants, the surface for floating plants, and above the surface for emergent plants).

To examine variation in producer community structure, I examined the distributions of producer groups from this survey. I examined this variation both at the whole community level with cluster analysis and at the individual functional group level to understand how variation in individual taxa contributed to whole community patterns and to align with previous studies (e.g., Scheffer et al. 2003). To obtain an index of phytoplankton abundance with the same range as the plant groups (necessary for multivariate analyses), I binned chlorophyll *a* levels by designating the three observations with values >100 µg/L as 100. To examine the distributions of individual functional groups, I created histograms and calculated several summary statistics (approach modified from Kelt and Meyer 2009). To identify discontinuous patterns, I characterized multimodality, since a discontinuous distribution (with many observations at the extremes and comparatively few intermediate observations) will exhibit more than one mode. For this, I used Hartigan and Hartigan's (1985) dip test with the diptest package in R 2.9.2, which computes the largest difference between the empirical distribution and a unimodal distribution for the observations. To evaluate for other shapes that would have many extreme values, I included measures of kurtosis, skewness, and departures from uniformity (Appendix Table A.1). These statistics would distinguish, for example, whether community types dominated by a particular

producer result from a non-normal distribution of that taxa with many high and low values (i.e. bimodality) or one high mode (i.e. a highly skewed, leptokurtic distribution).

To describe the whole community composition accounting for correlations among producers and to evaluate whether a finite number of community states were present, I used nonhierarchical cluster analysis. I chose the polythetic, divisive K-means procedure (20 iterations, Euclidean distances) in SYSTAT 10.0 using functional group abundances and binned phytoplankton levels. I chose the numbers of groups to include by plotting the within group sums-of-squares versus the number of clusters (similar to a Scree plot), and I chose the first number of clusters that explained a great amount of variation in the community (i.e. reduced within group sums-of-squares) while addition of the next cluster did not (McGarigal et al. 2000).

Spatial variation

To characterize whether variation in community composition was structured spatially, I used the producer data collected in late summer 2006 (described above) along with site location information. I performed a Mantel test using the vegan package (version 1.15-4) in R (Spearman's rank correlation, 999 permutations). For the community dissimilarity matrix, I designated the community states for each pair of wetlands (determined with cluster analysis) as matching (0) or not matching (1). For the distance matrix, I used the distance tool in ArcGIS 9.1 to measure distances between each pair of wetlands on aerial photographs from the U.S. Geological Survey.

Temporal variation

To characterize variation over time in plant composition, I compared abundances within and among seasons over several years (2005, 2006, 2008, and 2009; n=21-26). For the seasonal time series, plant community composition was recorded every 2-3 weeks throughout the 2005

growing season (Appendix A). For the annual survey data, visual estimates of plant abundances were collected as described above (except 2005, Appendix A). Late summer data were used for most sites, but earlier season (April-May) data were used for wetlands that were dry in mid-July. These sites had similar plant abundances throughout the season, such that the date for these sites made no difference in analyses.

To examine community stability, I used observations within and among seasons. For the seasonal time series, I examined the data graphically (see Appendix Figure A.1). For the interannual time series, community state for each wetland-year observation was determined from K-means cluster analysis with four clusters. (Resulting community types matched the cluster analysis of the 2006 data.) Community shifts between years were determined by identifying wetlands that changed community state between consecutive years (2005-2006, 2006-2008, and 2008-2009). If compositional changes were directional or cyclic, I would expect shifts between community states to occur in one sequence, yielding a strong association between initial community state and the type of shift occurring. I performed a 4 x 2 contingency table analysis to determine whether initial community state (four states as columns) was independent of whether or not a community shift occurred (two rows) (Zar 1999).

Drivers

To examine the role of possible drivers of these communities, I used abiotic data from the 2006 survey (n=35). I chose explanatory variables based on hypothesized mechanisms controlling producers, such as possible limiting resources in producer competition (nitrogen (N), phosphorus (P), N:P ratio, and light reaching the surface (i.e. forest canopy openness)) and possible physiological constraints of dominant groups (pH (Keddy 1976) and permanence (Euliss et al. 2004)). I also included conductivity, which determines water chemistry and reflects

hydrology of systems (Euliss et al. 2004), and dissolved oxygen, which can affect animal composition (thus grazing pressure) and biogeochemical cycling (Wetzel 2001). Finally, I included depth, because depth affects light attenuation, and because changes in water level have been shown to drive some aquatic plant communities (van der Valk 2005). Abiotic data were collected using standard methods (Appendix B).

To examine whether a discontinuous driver or unique taxa responses drove discontinuous community patterns, I used three multivariate techniques to study the relationship between the biotic and abiotic data from the 2006 survey. The techniques differ in how they model taxa responses to drivers (linear in discriminant function analysis (DFA), threshold in classification trees (CART), or unimodal in canonical correspondence analysis (CCA)). Thus, I compared my results from each to elucidate whether linear, simple threshold, or more complicated nonlinear patterns drove taxa (Figure 2.1). The techniques also differ in the kinds of predictors variables allowed. I used the eight continuous environmental variables in all analyses, but permanence was only used in CART because it was categorical. A site with incomplete water chemistry data was excluded from these analyses.

I explored whether linear-shaped drivers explained community state membership in DFA in SYSTAT. Total nitrogen and phosphorus were ln-transformed for this analysis. Although assumptions (equality of variance-covariance matrix, multivariate normality) were not fully met, DFA is acceptable as an exploratory technique in these circumstances (McGarigal et al. 2000).

To examine whether threshold-shaped responses or discontinuities in possible drivers explained differentiation of communities into alternate states, I used classification trees (CART analysis) in package mvpart (version 1.2-6) in R. This algorithm splits a dataset into several groups (nodes), choosing for each split a breakpoint in an independent variable to best classify

observations into community states. I set the minimum node sizes based on the least represented community state, specifying minima of six observations in a node that is split and two observations in a terminal node. Sites with incomplete data were excluded (n=28).

I used CCA to examine nonlinear responses to possible drivers. This technique forms linear combinations of the explanatory variables to explain the most variation in the biotic data. This technique performs well for unimodal and nonlinear monotonic responses of taxa to drivers (ter Braak and Verdonschot 1995). For CCA, I used visual estimates of each individual taxa (genera or families) rather than functional groups, since it is typically performed with species data (ter Braak 1986). I used only plant genera here, since macrophytes predominantly characterized the community states and I did not have genera-equivalent data for phytoplankton. I excluded observations with <10% cover of plants, since the analysis cannot be performed with sites having low abundances of all included taxa. I also excluded one site that was overweighted due to abundant rare species and one site (see Results and Appendix Figure A.1) with a late season change in composition (n=26). Total nitrogen and phosphorus were ln-transformed for this analysis to account better for nonlinear taxa responses to resources (Palmer 1993). I assessed significance of the constraints using permutation tests (with default settings), and I performed unconstrained correspondence analysis (CA) to verify that patterns seen in the constrained ordination based on abiotic variables aligned with the community variation seen in the cluster analysis (McGarigal et al. 2000) (Appendix Figure A.2). I performed ordinations using the vegan package in R. Default weighted average scores were used.

Results:

Characterizing composition

Producers showed evidence of discontinuous distributions at both the univariate and multivariate level. Individually, no producer groups showed evidence of uniform or normal distributions (Appendix Table A.1). For all three plant groups, the majority of wetlands had either minimal (0-20%) or abundant (80-100%) cover, with few wetlands between these extremes (Figure 2.2). The dip test suggested that the distributions of floating plants were multimodal (dip=0.113, $n=35$, $p<0.002$), while submerged and emergent vegetation distributions were skewed (submerged: $g_1=2.623$, $n=35$, $p<0.002$; emergent: $g_1=1.086$, $n=35$, $p<0.02$). Chlorophyll *a* values were skewed as well ($g_1=3.464$, $n=35$, $p<0.002$).

Within a multivariate framework, distinct community states were seen. Using cluster analysis, four and six groups explained large amounts of variation in the dataset (Appendix Figure A.3); I focus on the four cluster solution as the first point at which variation explained decreases (McGarigal et al. 2000; but see Appendix Figure A.4). With four clusters, groups emerge that are dominated by each plant functional group (Figure 2.3). These community states were dominated by (a) few mid-summer producers, (b) submerged plants, sometimes codominated by floating plants, (c) floating plants, sometimes codominated by phytoplankton, or (d) emergent plants (sometimes with moderate amounts of duckweeds and phytoplankton).

Spatial variation

The distribution of the communities was not spatially clustered; community state dissimilarity was not associated with distance (Mantel test, Spearman $r=-0.022$, $p=0.628$).

Temporal variation

Within a season, the sites exhibit persistence. Most communities did not change in plant

cover, or they followed a simple trajectory, in which they first increased and later decreased in plant abundance without major compositional changes (Appendix Figure A.1). Only one of the 21 wetlands had a distinct change in functional group dominance in mid-season (Appendix Figure A.1). However, among years I observed at least one shift in community state in nearly half (46.2%) of the wetlands (Table 2.1, Appendix Figure A.5). Of the wetlands that shifted, most shifted once, but four shifted twice. These wetlands always returned to their former state rather than shifting to a third community type. The likelihood of these shifts was not associated with a particular community type (4x2 contingency table analysis, $\chi^2=5.743$, $df=3$, $0.10 < p < 0.25$). Thus, these shifts did not represent directional changes between a series of community states from a primary to a climax state.

Drivers

Plant community state was partially explained by the environmental variables, but linear species responses did not solely drive these patterns. Linear-based DFA differentiated wetlands dominated by emergent vegetation well (89% of wetlands classified correctly), but overall only 62% of the wetlands were classified correctly in jackknifed classifications (Appendix Table B.1). Univariate scatterplots of the most strongly associated variables (light, pH, and depth) from the three analyses of drivers suggested threshold-shaped or nonlinear patterns underlie taxa responses (Appendix Figure B.1). In addition, univariate histograms suggested the drivers were not bimodal enough to generate the complex discontinuous patterns from gradual taxa responses alone (Appendix Figure B.2).

Allowing for nonlinear taxa responses improved differentiation of wetlands into community states based on abiotic variables. Classification trees sorted wetlands into community states well when permanence was included (Figure 2.4). In this tree, wetlands first

were divided by tree canopy cover. Of the low light sites, the more acidic ones were floating plant dominated, and the more neutral ones had few plants. Of the wetlands with more open canopies, most of the more temporary wetlands had emergent vegetation, while the more semipermanent ones were either floating or submerged plant dominated (with floating plants again more likely at lower pH values). The total misclassification of the tree was 13.8%, as compared to 62.1% with a null model assuming all observations should be classified as floating plant dominated (the most frequently observed state). The plant-dominated communities were differentiated well based on the first two axes in CCA without permanence (Figure 2.5). The first two CCA axes had reasonably high eigenvalues (first axis, defined most by dissolved oxygen, conductivity, and pH: 0.50, second axis, defined most by depth and light levels: 0.37, third axis (not shown): 0.20), and the model was significant based on permutation tests ($df=8,16$, $pseudo-F=1.693$, $permutations=799$, $p=0.031$). Wetlands dominated by submerged plants were associated with high pH and conductivity; sites dominated by floating plants were often deeper and more forested; and sites dominated by emergent vegetation were shallower and had more open forest canopies (Figure 2.5). Observations from the same community state grouped together in the CA based on genera as well (Appendix Figure A.2).

Discussion:

Multiple community states were observed in the producer communities of these wetlands, with each state defined by its dominant functional group of plants. These community states were not clustered over space and did not occur in a particular order over time. Despite the observation that many systems exhibited interannual variation, evidence for persistence within a season was strong. Finally, several abiotic drivers explained variation in community

composition, potentially driving expression of these states via nonlinear species responses. By evaluating these observations quantitatively using the framework from my conceptual model, I eliminated many hypotheses for multiple community states in this system. I found that alternative stable states and threshold-shaped responses are promising foci for future studies.

It is debatable whether these alternative community states are "stable," and this determination partially depends on how one defines stability and which data one considers most relevant (Connell and Sousa 1983, Paine et al. 1985, Grimm and Wissel 1997). Once they are established, these communities appear to be persistent within a season, lasting beyond the short lifespan of many dominant organisms (e.g., duckweeds). Yet, winter may reset these communities between years, similar to other systems in which disturbances can restart or change the path of succession (Sousa 1979, Petraitis and Dudgeon 2005). However, the wetland shifts were not associated strongly with particular community states and did not occur in any particular order, as would be expected in succession and cyclic succession. Thus, while other wetlands have been shown to exhibit successional dynamics after creation or disturbance (McMaster and McMaster 2001, Ray et al. 2001, Stroh et al. 2008), this does not appear as important in these established wetlands.

Although it is unclear what mediates transitions between states from these analyses, abiotic drivers may constrain the range of community states that can be expressed in a given system. Light, pH, and depth appeared important, with emergent plants most often dominating at higher light levels and lower depths, floating plants at lower pH values and higher depths, and submerged plants at higher pH values. Simple threshold responses of these taxa based on physiological tolerances are one explanation for these patterns. For example, *Lemna minor* is documented to be intolerant to high pH (Keddy 1976), and this species did not dominate any

wetlands with pH values above 6.6 in my 2006 dataset.

Yet, this does not preclude the possibility of more complicated mechanisms. Some of these variables may not represent direct causal relationships. For example, mechanistically, depth directly may affect germination of seeds in the spring (van der Valk 2005), or less directly it may affect attenuation of light through the water column (a property also influenced by phytoplankton density, turbidity, and colored dissolved organic compounds: Wetzel 2001). Also, pH may represent a self-stabilizing feedback mechanism (from the alternative stable states perspective), maintaining a community state rather than acting solely as a driver. For example, some producers photosynthesize enough in the day to increase pH greatly (Wetzel 2001), perhaps allowing submerged plants to exclude high pH intolerant taxa such as duckweeds.

In addition, drivers could be acting and/or interacting in more complicated ways. While CART can incorporate interactions among variables, it may not be able to account for the complicated two- and three-way interactions seen in some studies (e.g., Maestre and Reynolds 2006). Similarly, while CCA can detect a range of nonlinear patterns (ter Braak 1986, ter Braak and Verdonschot 1995, A. Yannarell, pers. comm.), it may not detect complicated ones perfectly, such as the hysteresis in alternative stable states theory. Other observations (e.g., the stability of these systems within a season and possible role of early season conditions in expression of states) suggest that alternative stable states may be worthy of more study in this system. To tease apart these possibilities, mechanistic-based statistical modeling of observational data or experiments (demonstrating self-stabilizing feedback mechanisms, mapping responses to manipulations of drivers, or forcing divergence to different states based on initial conditions) could be used (e.g., Schröder et al. 2005, Anderson 2008, O'Farrell et al. 2009).

As humans continue to accelerate changes in slow drivers such as climate, nutrient

cycling, and disturbance, even well studied ecosystems increasingly will exhibit novel structures and behaviors (Chapin et al. 2006, Fox 2007, Seastedt et al. 2008). Since thresholds and nonlinear behaviors frequently are implicated with these changes, we increasingly will be challenged to understand the causes of discontinuous variation in community composition in both research and management. Part of the careful evaluation of hypotheses explaining this variation relies on conducting well-designed experiments. Yet, making solid conclusions as we find our way through this maze of hypotheses also relies on a strong observational underpinning at the outset of our studies. While ecologists often consider a variety of hypotheses as they begin new studies, the proposed formal framework may help us navigate the wide range of possible mechanisms.

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Figures and Tables:

Table 2.1. Interannual variability in plant community structure in temporary and semipermanent wetlands in Michigan, USA. Shown are the observed numbers of community shifts (with percentages of all year transitions), grouped by initial community state. Numbers of community shifts expected were based on contingency table analysis, assuming no association between initial community state and the likelihood of a shift. Community shifts and initial community states were defined using K-means cluster analysis of all wetland-year observations (see Methods for details).

	n	Observed	Expected
<i>By community state in first year of transition:</i>			
Few producers	31	3 (9.7%)	6.99
Submerged dominated	8	2 (25.0%)	1.80
Floating dominated	19	6 (31.6%)	4.28
Emergent dominated	13	5 (38.5%)	2.93
<i>Total number of community shifts</i>	<i>71</i>	<i>16 (22.5%)</i>	

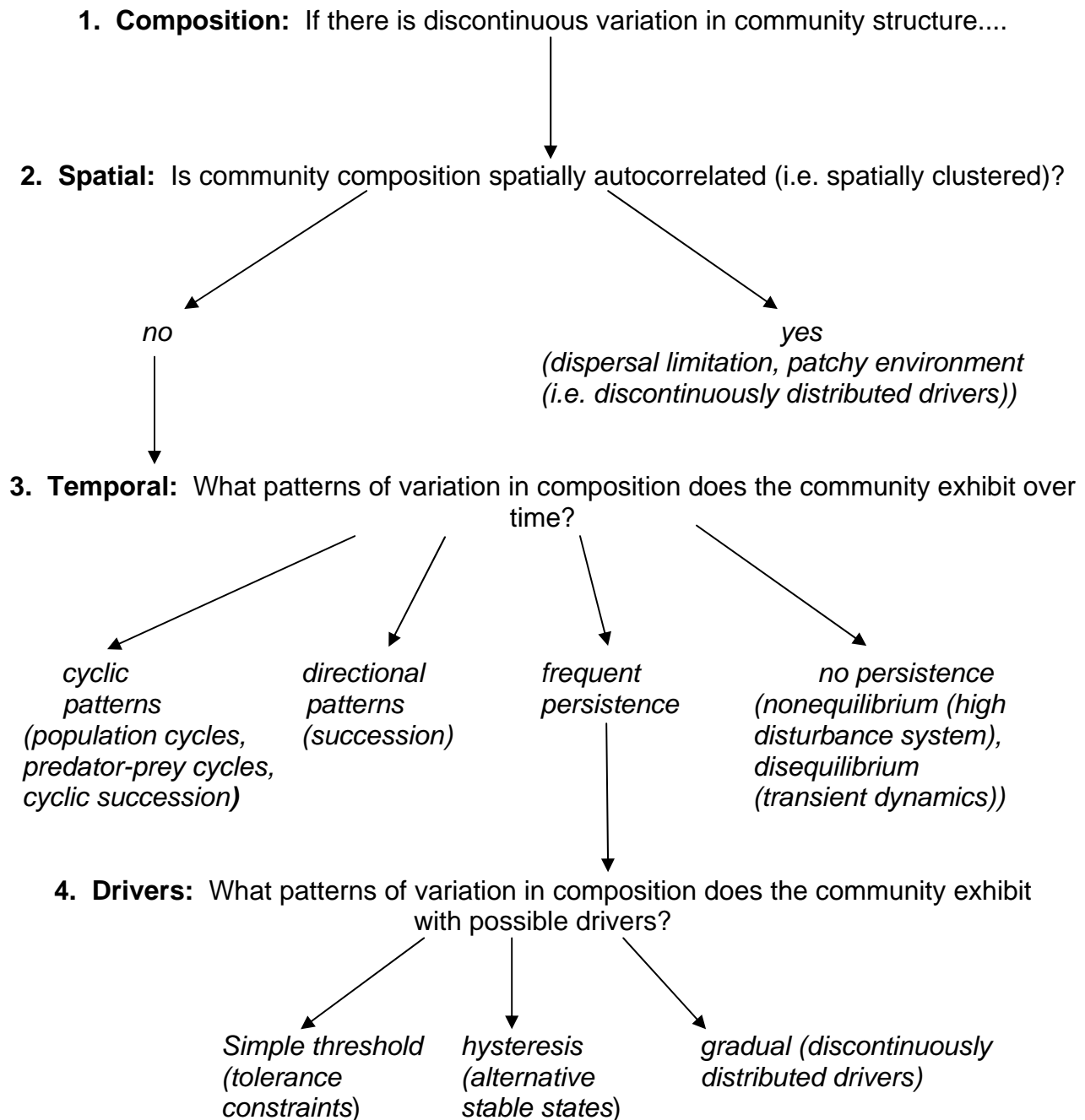


Figure 2.1. Framework for distinguishing possible causes of discontinuous variation in community composition in ecological systems. Questions I ask are in plain type, while observations and some possible explanations based on them are in italics (with explanations in parentheses).

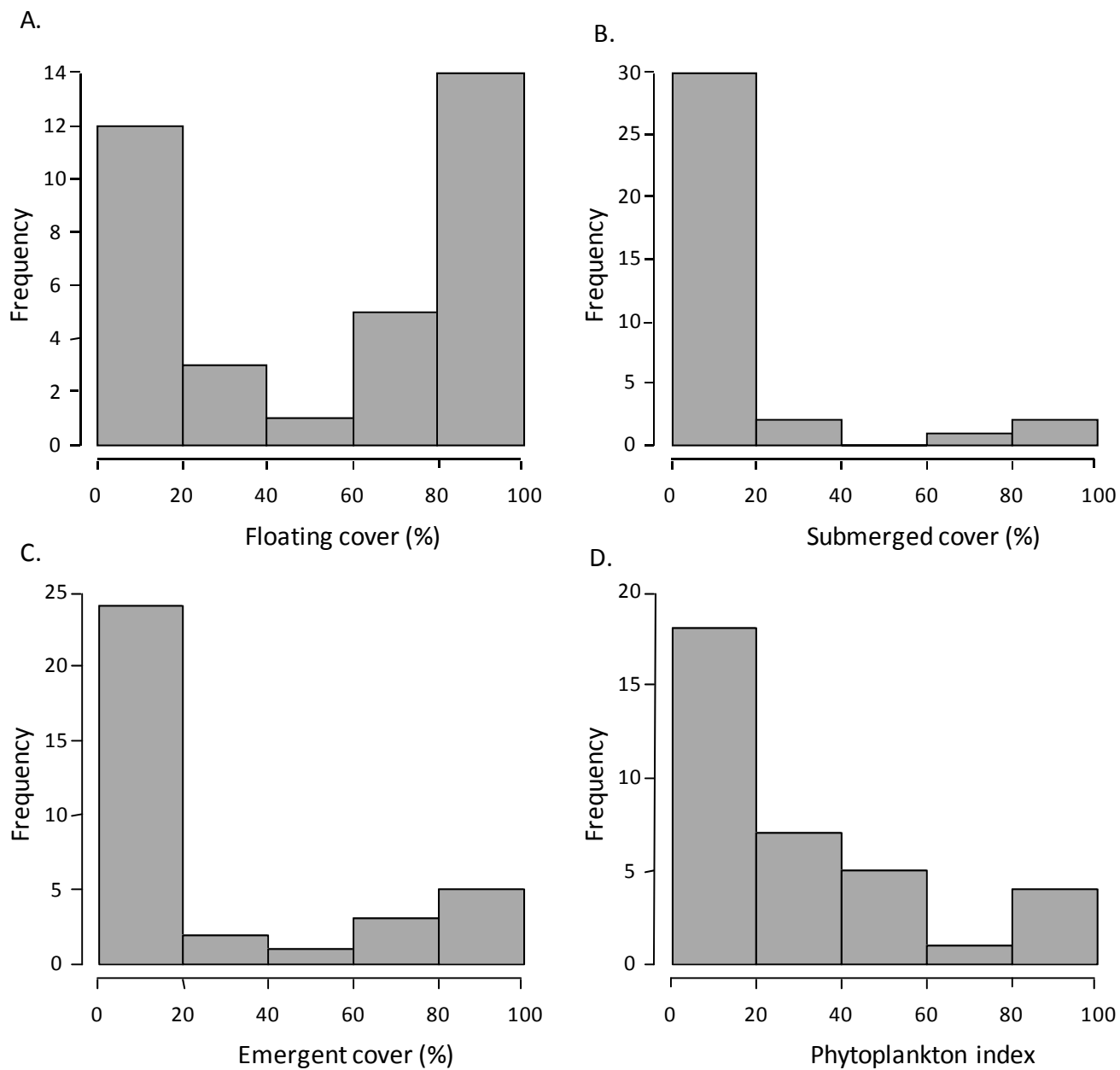


Figure 2.2. Histograms of each producer functional group in a survey of wetlands in southwest Michigan, USA (n=35). As an index of phytoplankton abundance, chlorophyll *a* levels are converted to a range of 100 by binning all values $\geq 100 \mu\text{g/L}$.

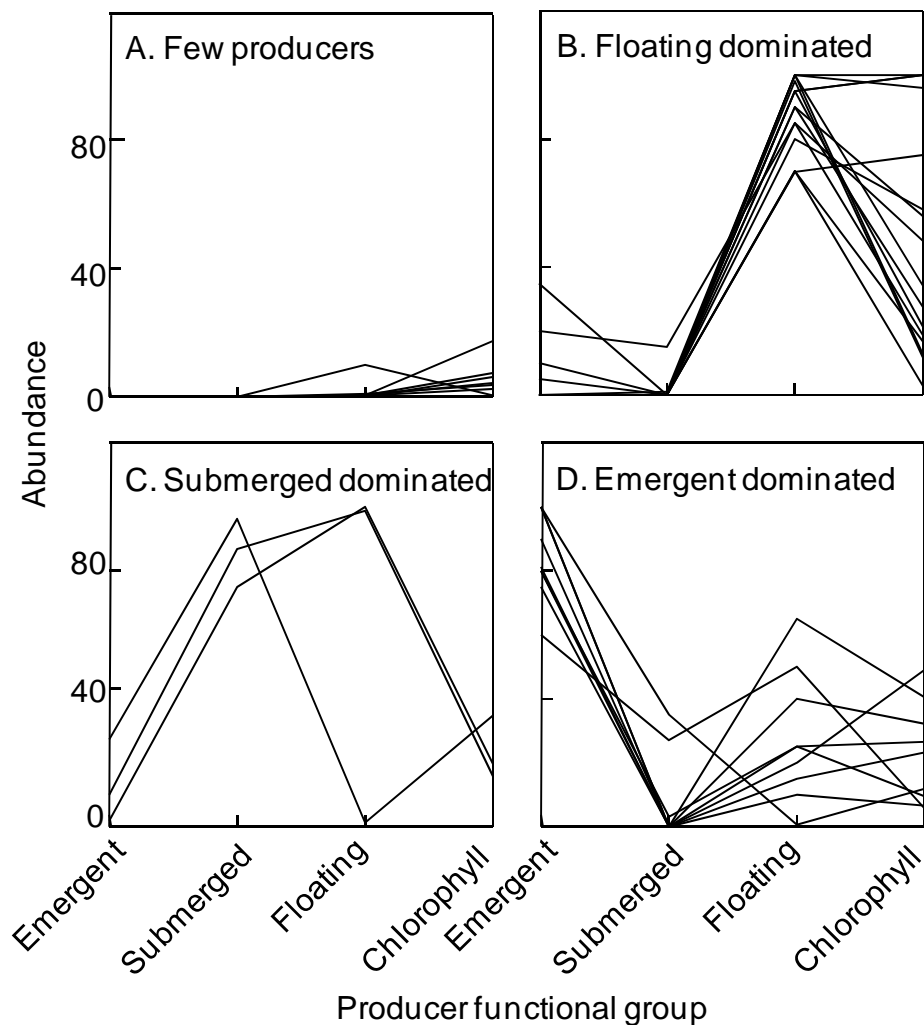


Figure 2.3. Cluster analysis of producer communities of wetlands in southwest Michigan, USA. In the parallel coordinates plots, each line represents the abundances of each producer functional group in one wetland observation, and each panel represents one cluster (community state) of wetlands identified in the analysis. Four clusters were identified, distinguished by dominance of: (A) few producers in mid-late summer ($n=7$), (B) floating plants ($n=16$), (C) submerged plants ($n=3$), and (D) emergent plants ($n=9$). Plant abundances are percent cover estimates; chlorophyll *a* levels were converted to a range of 100 by binning all values $\geq 100 \mu\text{g/L}$.

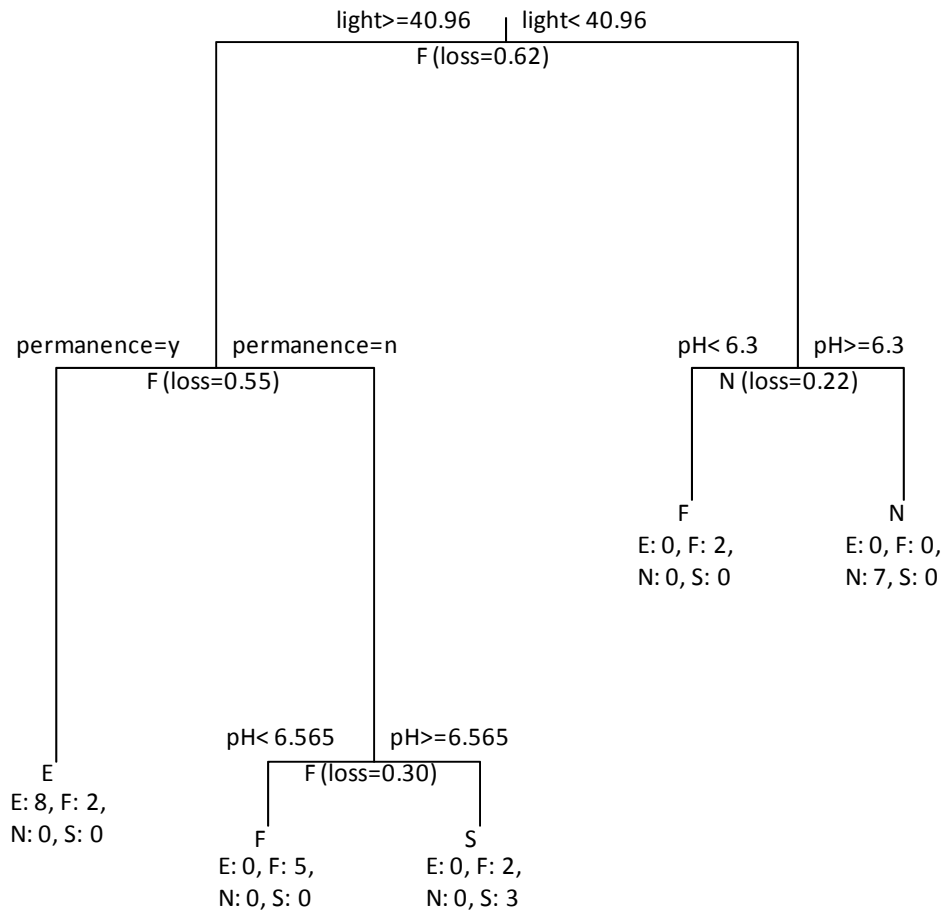


Figure 2.4. Classification tree (CART) for producer communities of wetlands in late summer 2006 in southwest Michigan, USA. All nine explanatory variables were used (depth, dissolved oxygen, conductivity, pH, light, TN, TP, TN:TP, and permanence), and only observations with complete data were included (n=29). Each node in the tree is labeled with which community state is most abundant in it, and the number of observations of each community state is shown for terminal nodes. States are abbreviated as: emergent (E), floating (F), few (N), and submerged (S) plant dominance. At each intermediate node, I also included how the node was split and the expected loss (proportion of observations misclassified if further splitting is not attempted). Light is the percentage of light reaching each wetland's surface (i.e. canopy openness). For permanence, I scored sites based on whether they dried at the end of a moderately wet year ("y") or not ("n") (see Appendix B for details).

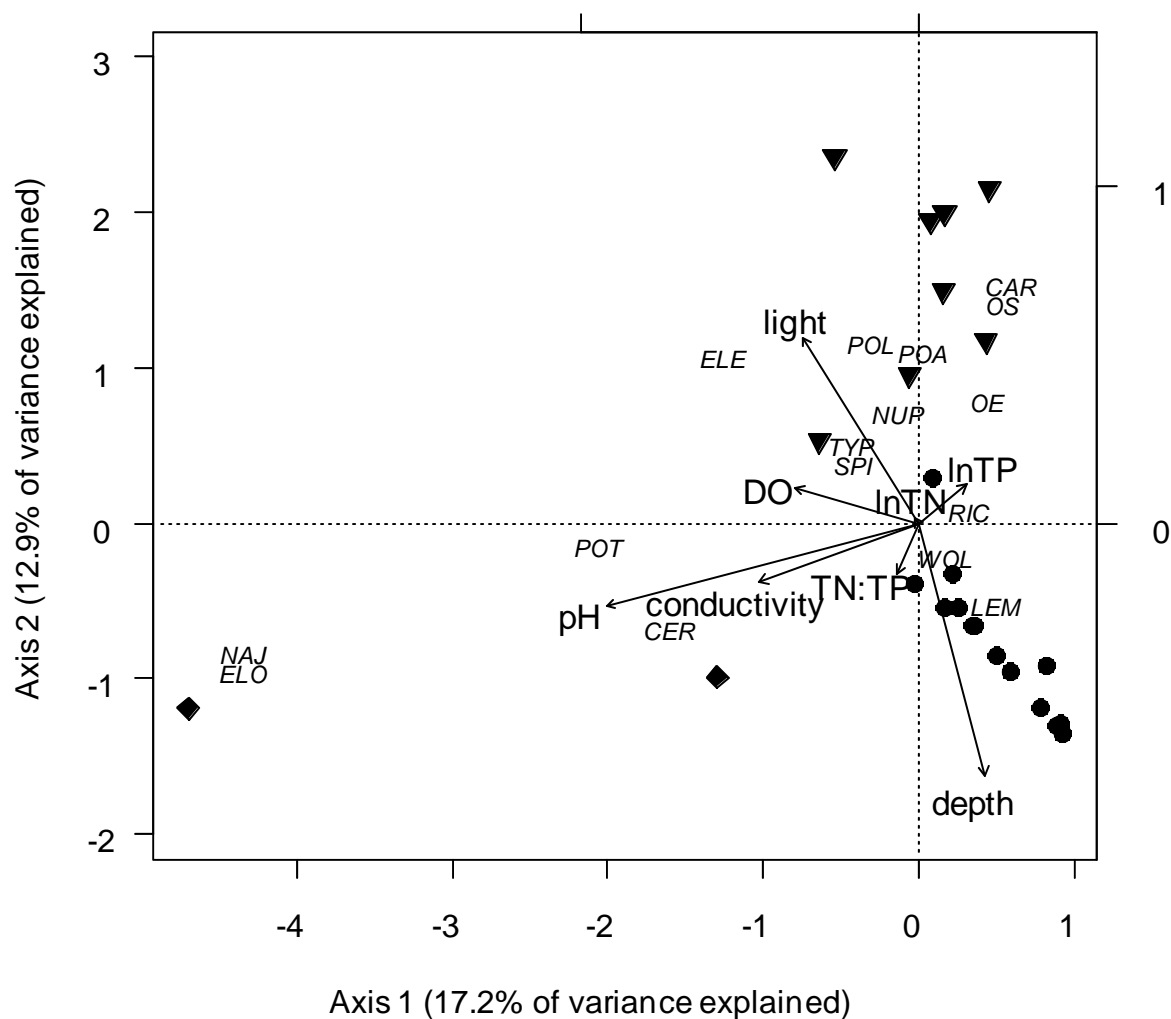


Figure 2.5. Triplot from canonical correspondence analysis (CCA) of 16 taxa vs. environmental variables (n=26 plant-dominated wetlands, late summer 2006). Sites scores (symbols), taxa scores (3-letter abbreviated names), and environmental variables (arrows) are shown. Site scores are weighted averages, with symbols representing community state: dominated by floating (circles), submerged (diamonds), or emergent (triangles) vegetation. Environmental variables included are depth, dissolved oxygen (DO), conductivity, pH, percent of light reaching surface (light), total nitrogen:total phosphorus ratio (TN:TP), and ln-transformed total nitrogen and phosphorus (lnTN and lnTP, respectively). See Appendix Figure A.2 for full taxa names.

CHAPTER 3

THE ROLE OF NUTRIENTS IN REGULATING THE STRUCTURE OF AQUATIC PLANT COMMUNITIES*

Abstract:

Blooms of free-floating plants in freshwater systems have attracted the interest of scientists and managers alike. I evaluated the role of nitrogen (N), phosphorus (P), and N:P ratios in driving these plants' dominance in ponds in Michigan, USA. I found that floating plants exhibited strong patterns with total N, total P, and N:P ratios in surveys of ponds. Exploring these relationships further, I manipulated all three variables in enclosures in two experimental ponds with floating and submerged plants. I found N supply rates to drive duckweed abundances more strongly than P and N:P after 20 days. Yet, other factors affected duckweed abundances as well. Duckweeds often were associated with acidic pH levels. Also, composition of submerged plants (especially abundances of *Potamogeton pusillus-pectinatus*) affected whether floating plants could codominate systems with submerged plants. This highlights the need to understand better the complex interactions between all of these and other potential drivers of producer community dynamics.

Introduction:

Scientists and managers alike have been challenged to identify the conditions leading to free-floating plant dominance in lakes and smaller water bodies. Floating plant blooms can change phytoplankton composition and shade out submerged macrophytes (Portielje and Roijackers 1995, Scheffer et al. 2003, Morris et al. 2003, O'Farrell et al. 2009). Further, these

*Spencer R. Hall contributed to the collection and analysis of the survey dataset.

blooms can change habitat structure, water chemistry, and animal diversity in both natural and constructed water bodies (Hillman 1961, Meerhoff et al. 2003, Scheffer et al. 2003, Villamagna and Murphy 2010). While a suite of possible causes of floating plant blooms has been identified in previous studies (e.g., nitrogen loading, phosphorus loading, hydrology changes, climate change: Portielje and Roijackers 1995, Scheffer et al. 2003, Feuchtmayr et al. 2009, O'Farrell et al. 2009), determining their relative importance in individual systems with differing ecosystem structures is challenging.

Floating plants often are associated with eutrophic conditions, but the question of which nutrient most strongly drives floating plant growth is less clear. Nitrogen (N) and phosphorus (P) long have been considered limiting nutrients in aquatic systems (e.g., Hasler 1947, Lee 1973, Schindler 1974, Lund 1974), and co-limitation of both nutrients in natural systems is frequent as well (Elser et al. 2007). Both N and P have been implicated to cause increases in floating plant abundances through different mechanisms (Scheffer et al. 2003, Lucassen et al. 2005, Smolders et al. 2006, Szabo et al. 2010), and individual populations often exhibit growth rates positively correlated with N and P availability. For example, Kufel et al. (2010) found that lesser duckweed *Lemna minor*, a cosmopolitan free-floating plant species, exhibited increasing growth rates with increased ammonium and soluble reactive P.

The relative concentrations of nutrients (i.e. N:P ratio) may play a role in determining which primary producers dominate as well, as emphasized in ecological stoichiometry theory (Tilman 1977, Tilman 1982, Sterner and Elser 2002). Species can exhibit differences in their relative competitive abilities under limitation by different resources (Koerselman and Meuleman 1996), since species can have differing abilities to acquire different resources. In previous studies of herbaceous aquatic plants, the signature of a transition from N to P limitation has been

determined, where communities are N limited below plant tissue N:P of 14:1 but P limited when plant N:P ratios are above 16:1 (Koerselman and Meuleman 1996, Bedford et al. 1999). If these plant tissue ratios reflect N and P availability in the environment, then the relative differences in nutrient availability could drive changes in competitive outcomes between species. This would lead to communities having shifts in composition across this transition.

Yet, other factors may mediate floating plant responses to increases in nutrient availability. One factor is the composition of accompanying producers, since floating plants compete with other functional groups of producers. Scheffer et al. (2003) suggested that floating plants and submerged plants compete for nitrogen and other limiting resources (e.g., light), such that availability of multiple resources may mediate competition between these groups.

Filamentous algae may form floating beds or coat aquatic plants as well (i.e. as epiphyton and metaphyton: Phillips et al. 1978, Roijackers et al. 2004, James et al. 2006). Since these algae also may respond to increased nutrient levels, there is potential for nutrient enrichment to drive algal increases that shade out the floating plants. This would cause lower rather than higher floating plant cover. Another factor that can constrain floating plants is pH tolerances. For example, *L. minor* tends to occur in mildly acidic conditions (Keddy 1976), and other lemnids tend to occur in acidic to neutral conditions as well (Hillman 1961, McLay 1976, Chapter 2, Appendix B). This means that even extremely high nutrient concentrations would not necessarily lead to high *L. minor* populations in basic conditions, but instead may lead to dominance of species tolerant of higher pH levels.

Here, I focused on the interplay of N and P in controlling floating plant dynamics in temporary and semipermanent wetlands (hereafter, ponds) in Michigan, USA. These ponds offered ideal systems to use both observational and experimental approaches. First, I examined

patterns in the dominance of floating plants with nutrient levels in an observational dataset spanning broad nutrient availability gradients. Second, I tested the effects of manipulating the supply rates of N, P, and N:P ratios on floating plant abundances in a field mesocosm experiment. These data together allowed me to examine which nutrients (N, P, and/or their ratio) affected floating plant abundances and to evaluate these responses in context of abiotic conditions and submerged plant abundance and composition.

Methods:

Observational work:

Ponds were surveyed in July-August 2000 to study how producer community composition varies with nutrient levels (n=38 ponds). Here, I present methods relevant to this study; further details may be found in Hall et al. (2005). Ponds were located in Barry, Kalamazoo, and Mason Counties in Michigan, USA. Macrophyte and microphyte cover were recorded by species as visual estimates (percent cover of each whole pond). These estimates were pooled by functional group (classifying each species as floating, submerged, or emergent, defined by their position in the water column: Chapter 2). The sum of both functional groups sometimes exceeded 100 percent, since each functional group had biomass concentrated on different planes of wetlands (i.e. the basin for submerged plants and the surface for floating plants). Water samples were collected for water chemistry analyses. Total N (TN) was measured by persulfate digestion and second derivative spectroscopy (Crumpton et al. 1992). Total P (TP) was measured using the ascorbic acid method after persulfate digestion (Eaton et al. 2005). A U-10 multi-probe (Horiba, Ltd., Kyoto, Japan) was used to measure pH. I examined

the relationships between floating and submerged plant abundances to total N, P, N:P, and pH levels graphically.

I performed K-means cluster analysis in SYSTAT 10.0 to identify communities dominated by floating or submerged plants. I ran the analysis on the abundances of submerged and floating vegetation with 2-6 groups. Three groups best explained the variation in the dataset (McGarigal et al. 2000), exhibiting dominance of (1) floating plants (abundances $\geq 80\%$ floating, $\leq 30\%$ submerged), (2) submerged plants ($\geq 55\%$ submerged, $\leq 5\%$ floating, or (3) neither group ($\leq 50\%$ floating, $\leq 35\%$ submerged vegetation).

I examined the responses of floating and submerged plants to N, P, N:P, and pH for nonlinear patterns. Thresholds in these responses were identified with classification trees (CART technique) using package mvpart (version 1.2-6) in R 2.9.2. I limited the dataset for this analysis to the 20 ponds that exhibited floating or submerged plant dominance. I used community state as the dependent variable and each abiotic variable individually (N, P, N:P, and pH) as the independent variable. Cross-validation with the one s.e. criterion was used for tree pruning.

Field experiment:

I manipulated N, P, and N:P ratios to examine the responses of floating plants to different nutrient supplies. This work was conducted in two experimental ponds (ponds 13 and 14) at the Experimental Pond Facility (W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, USA). I performed the experiment in watertight cylindrical enclosures (~1 m wide) open to the sediment and the atmosphere. These ponds have dense submerged plant

communities with few floating plants most years; I added floating plants to the enclosures to examine their responses to nutrients.

Based on patterns in the field data (Figure 3.1) and previous work highlighting the possible role of N (e.g., Scheffer et al. 2003), I predominantly focused on mapping producer responses to N availability. To accomplish this, I used five N addition treatments, increasing from ambient (adding ultrapure water) to hypereutrophic (aiming to increase water column TN levels to 5000 $\mu\text{g/L}$). Phosphorus was added concurrently with a target N:P ratio of 14.6 (the average for all pond observations in two datasets: survey datasets presented here and in Chapter 2). I assumed an initial water column TN concentration of 800 $\mu\text{g/L}$ and TP concentration of 42 $\mu\text{g/L}$ based on water samples collected from the ponds 9 June 2008.

I also tested the role of P and N:P on plant abundances. To incorporate these manipulations into the treatment scheme, I offset the target N:P ratios in the second and third N treatments, such that treatment 2 was proportionately high in P (N:P ratio of $\sim 7:1$) and treatment 3 was proportionately low in P (N:P ratio of $\sim 25.5:1$) (Figure 3.2). These treatment levels were chosen to set the low target N:P within the range of duckweed-dominated ponds (3.42-22.87; mean = 11.15, n = 13 ponds) and the high target N:P near the range of submerged plant-dominated ponds (25.88-42.67, mean = 32.27, n = 6 ponds) of the field data presented here. However, these values were not selected to match the field averages, since I also constrained the treatment 2 P target to match the treatment 4 P target and the treatment 3 P target to the double of ambient P levels (Fig 3.2). The five treatments were randomly assigned within rows using a complete block design (3 replicates per pond).

I installed the enclosures and conducted initial submerged macrophyte surveys 19-22 May 2008. I drained the ponds for ~ 24 hours to record visual estimates of submerged cover (the

proportion of the area within each enclosure covered by vegetation, with stems against the ground). After refilling the ponds to ~1 m deep, the enclosures (vinyl coded wire mesh cages lined with 6 mil plastic sheeting) were driven into the sediment by hand.

All subsequent additions and sampling were carried out by small watercraft to prevent disturbance near the enclosure bottoms. Nutrients were added beginning 17 June 2008 using concentrated solutions of NaH_2PO_4 and NaNO_3 in ultrapure water. To account for small depth differences among enclosures, nutrients for each enclosure were aliquoted individually, with additions adjusted for water volume inside each enclosure on the day of the addition. Additions were mixed into the enclosure water by filling and dumping a 1.9-L pitcher. I continued to add nutrients (10% of the original nutrient targets) weekly during the experiment.

Equal amounts of duckweed were added into each enclosure (19-22 June; day 1 of the experiment is considered 21 June 2008). Duckweeds (predominantly *Lemna minor*, with smaller amounts of other species) were collected from eight natural ponds. Duckweeds were weighed into aliquots after mixing, removing invertebrates and detritus, rinsing, and blotting gently with paper towels. Aliquots (200.0 \pm 0.2 g total, intending to cover 50% of surface area) were added to each enclosure and allowed to spread naturally.

During the experiment, I monitored floating plant cover weekly by digitally photographing the enclosure surfaces. Percent cover of floating taxa was determined from the photographs using image analysis software. The area of interest was designated manually, contrast levels of the grayscale photos were adjusted to account for differences in exposure, and pixels were classified as floating taxa or open water using thresholds in Adobe Photoshop 6.0 and MATLAB R2009a. Since floating algal beds and submerged plants with leaves on the

surface were detected with these methods, I subtracted visual estimates of these two groups from the floating taxa estimates before analysis.

At the end of the experiment, filamentous algae was removed from the surface of each enclosure (25-29 August = days 66-70), dried, and weighed. Also, pH levels were taken using a Hydrolab unit (Hach Co., Loveland, CO, USA) (21-22 August = days 61-62). The ponds were drained, and submerged macrophyte abundances were estimated as described above (29 August 2008 = day 70). I maintained my initial survey methods for the submerged plants to be able to compare my initial and final abundance estimates.

I evaluated the responses of floating plant abundances to treatments using ANOVA models run with PROC MIXED in SAS 9.2 with Kenward-Roger degrees of freedom computation. I analyzed the experiment as a generalized randomized block design, assessing the effects of pond (random block), treatment (fixed), and their interaction on floating taxa cover. I performed these ANOVA models at individual dates at the end of their initial growth response (day 20) and near the end of the experiment before enclosure effects were strong (day 42). Duckweed proportions were arcsine-transformed prior to the individual date analyses to conform to normality assumptions. For day 20, I used a compound symmetry intraclass correlation model to account properly for a negative pond x treatment covariance parameter estimate (i.e. to allow for correlated errors: Littell et al. 2006). On day 42, this interaction was dropped from the final model because it was not significant.

Contrast statements were used to pull apart the effects of nutrient treatments. To test for the effect of N supply, I compared the treatments that did not have N:P ratio manipulations (treatments 1 vs. 4 vs. 5). To test the effects of P supply, I compared the treatments with the lowest, highest, and most intermediate P additions (treatments 1 vs. 5 vs. average of 2 and 4,

respectively; see Figure 3.2). Finally, to test the effect of N:P, I compared the two offset N:P treatments to those with the targeted intermediate N:P supply rate (treatments 2 vs. 3 vs. average of 1 and 4, excluding treatment 5 to use a smaller range of nutrient loadings).

Finally, I examined whether the compositional differences in submerged plants that I observed in the beginning of the experiment were associated with any differences in final floating or submerged taxa cover. I tested whether submerged taxa abundances were different between the experimental ponds before the experiment and whether this led to differences at the end of the experiment using separate variance t-tests in SYSTAT 10.0.

Results:

Field surveys showed relationships in the responses of floating plants to total nitrogen (TN), total phosphorus (TP), and TN:TP. For TP and TN:TP, a threshold-like transition was seen for both floating and submerged plant abundances, with each plant group only dominating above or below a given level (Figure 3.1). At the TP and TN:TP levels that floating plants never dominated, submerged plants sometimes did. However, the relationship of floating plants to TN was qualitatively different. Intermediate TN levels had observations of both community states around the threshold identified by the CART analysis, but duckweeds always dominated at the highest TN and never dominated at the lowest TN levels (Figure 3.1). In addition, floating plants only dominated in acidic conditions (Figure 3.1).

Examining the responses of floating plants in the experiment, nutrients significantly affected floating plants during the initial growth response (ANOVA day 20, Table 3.1). This response was most likely driven by N based on the preplanned contrasts (ANOVA day 20, Table 3.1), with enclosures being increasingly likely to have high floating plant cover with higher N

levels (Figure 3.3A). The contrast for the effect of P was significant as well, but these treatment differences appeared driven by the high duckweed cover in the highest N treatments more so than a consistent trend with increasing P (Figure 3.3A). The main effect of nutrient treatment was not significant by day 42 (Table 3.1).

Despite no effect of pond on duckweed abundances (Table 3.1), other strong producer differences by pond were observed. For the submerged plants, abundances at the end of the experiment differed by pond (separate variance t test, $df = 13.6$, $t = 5.65$, $p < 0.001$; Figure 3.4A). In pond 13, submerged plant cover increased during the experiment, such that every enclosure was filled with submerged plants at the end of the experiment (total cover $99 \pm 1\%$, mean ± 1 s.e.; Figure 3.4B). In contrast, in the pond 14 enclosures, submerged plant abundances had little change or were lower than they started (Figure 3.4B). This may be explained by initial submerged plant cover, since this variable differed between the two ponds before the experiment started (separate variance t test, $df = 26.7$, $t = -8.914$, $p < 0.001$). Differences in species composition were even more pronounced. Initially, most of the enclosures in pond 13 were more sparsely populated with near monoculture of *Potamogeton pusillus/pectinatus* (total submerged cover of $47 \pm 2\%$, mean ± 1 s.e.; 80% of this submerged cover was *Potamogeton pusillus/pectinatus*, $n=15$), while pond 14 enclosures had more abundant and diverse mixes of four submerged species (total submerged cover $85 \pm 3\%$; 0% of this cover was *Potamogeton pusillus/pectinatus*, $n=15$).

Floating taxa identity shifted in the latter part of the experiment. By day 42, pond 14 enclosures predominantly had filamentous algal beds (Figure 3.5A), while pond 13 enclosures predominantly had *Wolffia* sp. This shift away from *Lemna minor* may have resulted from

increases in pH during the experiment, since pH was quite basic in the enclosures by the end of the experiment (9.39 ± 0.11 , mean \pm 1 s.e., $n = 29$, Figure 3.6).

Discussion:

Field patterns suggested that nitrogen (N), phosphorus (P), and N:P ratios were all possible drivers of pond producer communities. When I tested the relative importance of these nutrients experimentally, treatment differences in producer abundances observed in the first part of the experiment were explained better by N availability than by P or N:P availability. While N and P limitation have been documented to occur equally across many ecosystems (Elser et al. 2007), this result lends support to previous suggestions that N in particular may control floating plant dominance in shallow lakes and smaller water bodies (Scheffer et al. 2003). However, studies have found frequent co-limitation of multiple nutrients in aquatic systems (Barko and Smart 1986, Elser et al. 2007), and in these observational data, P was associated with plant community composition as well. Thus, future studies may be useful to explore situations in which P plays a stronger role in determining plant community composition.

Both species composition and abiotic conditions were important in determining the responses of floating plants in the experiment as well. Floating plants appeared to have strong relationships to nutrients in the observational data, yet a nutrient treatment effect was absent by day 42. This may be explained by the observation that the dominant species of floating plants in the observational dataset (*Lemna minor*) did not persist in the experimental enclosures during the second half of the experiment. This was most likely simply due to physiological intolerance of *L. minor* to higher pH values (Keddy 1976, Roijackers et al. 2004, Chapter 2, Appendix B). Instead, pond 14 enclosures developed floating algal beds, and pond 13 enclosures became

dominated by *Wolffia* sp., of which one congener has been shown to grow better than *L. minor* at high pH levels (McLay 1976). Thus, it appears that pH strongly affected species composition of floating plants, and species composition in turn affected plant responses to the nutrient additions. While one might be surprised that *Wolffia* sp. did not exhibit a strong positive responses to nutrients, I have observed *Wolffia*-dominated ponds with lower total N than measured in ponds dominated by *L. minor* (Chapter 4).

In contrast to the observational data, many pond 13 enclosures had high abundances of both floating and submerged plants in the experiment. In the field dataset, floating and submerged plant abundances often were inversely correlated: ponds dominated by floating plants were never dominated by submerged plants (and vice versa). However, in another field survey of ponds in the same areas, I observed two ponds codominated by both functional groups of plants (Chapter 2). The submerged species that dominated pond 13 in the experiment (*Potamogeton pusillus/pectinatus*) is one of the few species I have observed to codominate ponds in my survey work (Chapter 2, S. Smith, pers. observation). This particular species has been observed to coexist with floating and emergent plant species in an Iowa lake as well (Egertson et al. 2004). Strong shade tolerance of *P. pectinatus* has been noted in past studies comparing its competitive abilities to submerged charophytes (van den Berg et al. 1998, 1999). This is because this species has a higher leaf canopy in the water column than some other submerged taxa (van den Berg et al. 1998, 1999). This architecture may allow it to compete with floating plants differently than other submerged species.

These contrasts between the observational and experimental data suggest that species composition can affect floating and submerged plant dynamics. Using functional groups to characterize community structure often helps to decipher community dynamics, yet classifying

plants by functional group based on growth form did not capture the full suite of dynamics observed here. These results suggest complex interactions among nutrients, species composition of other producers, and abiotic conditions in driving floating plant responses (as seen in Chapter 2). Further studies of these complex interactions may help to identify situations in which studying producer dynamics at lower taxonomic levels can lead to greater understanding of community structure.

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Figures and Tables:

Table 3.1. Responses of floating plants to nutrient additions in the enclosure experiment using ANOVA at the end of the initial growth phase (day 20) and later in the experiment (day 42). Floating cover proportions were arcsin-transformed prior to analysis to fit test assumptions. On day 20, a compound symmetry (CS) intraclass correlation model was used. (Abbreviations used: Num DF = numerator degrees of freedom, Den DF = denominator degrees of freedom, F = F-statistic, p = p-value.)

A. Random effects

DAY 20	Covariance parameter estimate \pm 1 s.e.	DAY 42	Covariance parameter estimate \pm 1 s.e.
Pond	0.03 \pm 0.05	Pond	0.00 \pm 0.00
CS (Pond x treatment)	-0.01 \pm 0.01	Pond x treatment	
Residual	0.04 \pm 0.01	Residual	0.04 \pm 0.01

B. Fixed effects

DAY 20	Num DF	Den DF	F	p	DAY 42	Num DF	Den DF	F	p
Treatment	4	4.16	10.60	0.02	Treatment	4	23.1	1.03	0.41
Contrast N (1 vs. 4 vs. 5)	2	4.42	12.35	0.02					
Contrast N:P (2 vs. 3 vs. 1&4)	2	4.09	5.51	0.07					
Contrast P (1 vs. 2&4 vs. 5)	2	4.48	7.21	0.04					

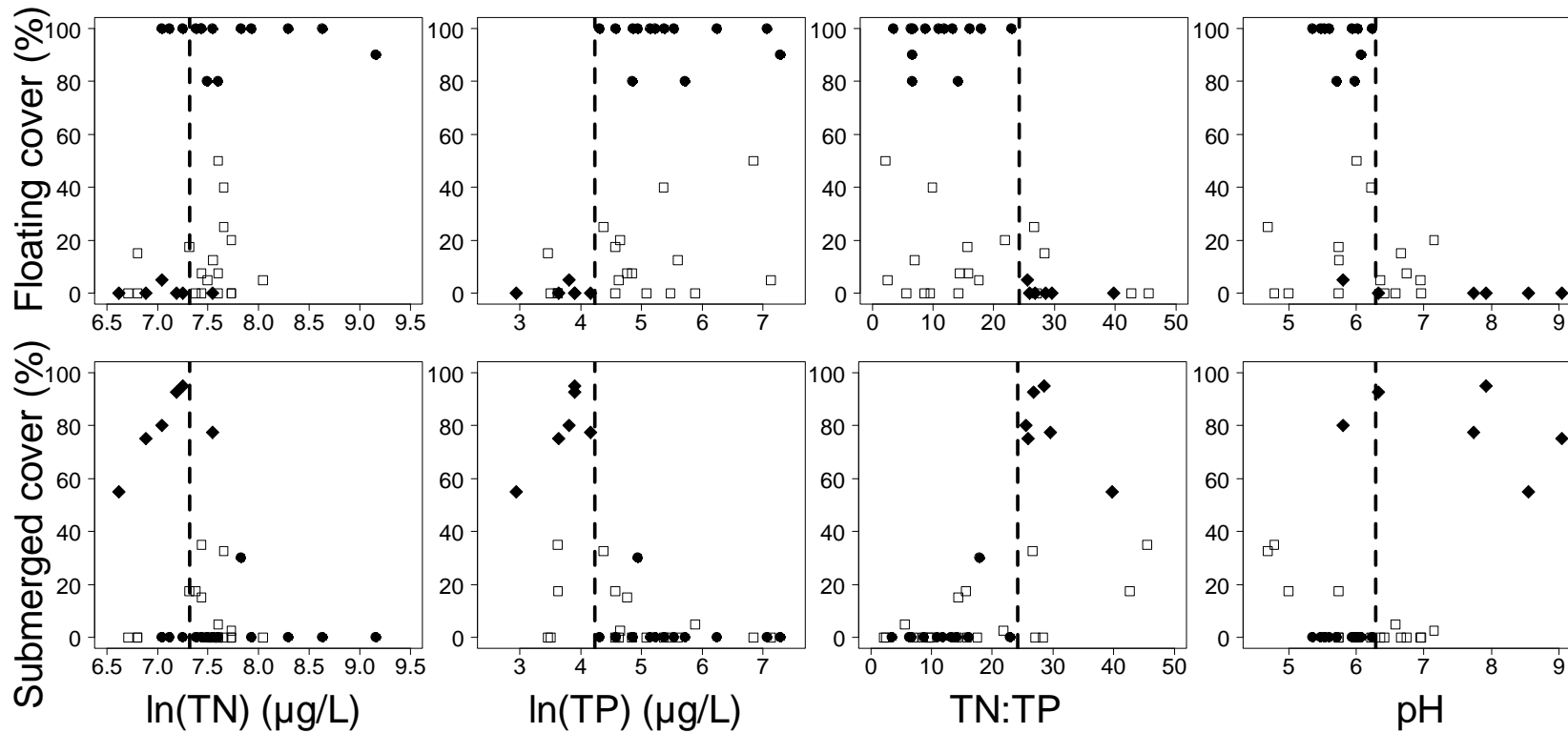


Figure 3.1. Relationship of floating and submerged plant abundance (percent cover) with nitrogen (TN), phosphorus (TP), their ratio (TN:TP), and pH in 38 temporary and semipermanent ponds in Michigan, USA. Total N and P levels are \ln -transformed. Symbols denote community state, as identified from K means cluster analysis: community states dominated by submerged plants (diamonds), floating plants (circles), or neither plant group (squares). Floating and submerged plant dominated community symbols are filled for emphasis. Dashed lines denote thresholds identified from classification tree (CART) analysis of 20 floating- and submerged-dominated ponds (TN 1506 $\mu\text{g/L}$ with 4 misclassifications; TP of 69.2 $\mu\text{g/L}$ with 0 misclassifications; TN:TP of 24.2:1 with 0 misclassifications; pH of 6.28 with 1 misclassification).

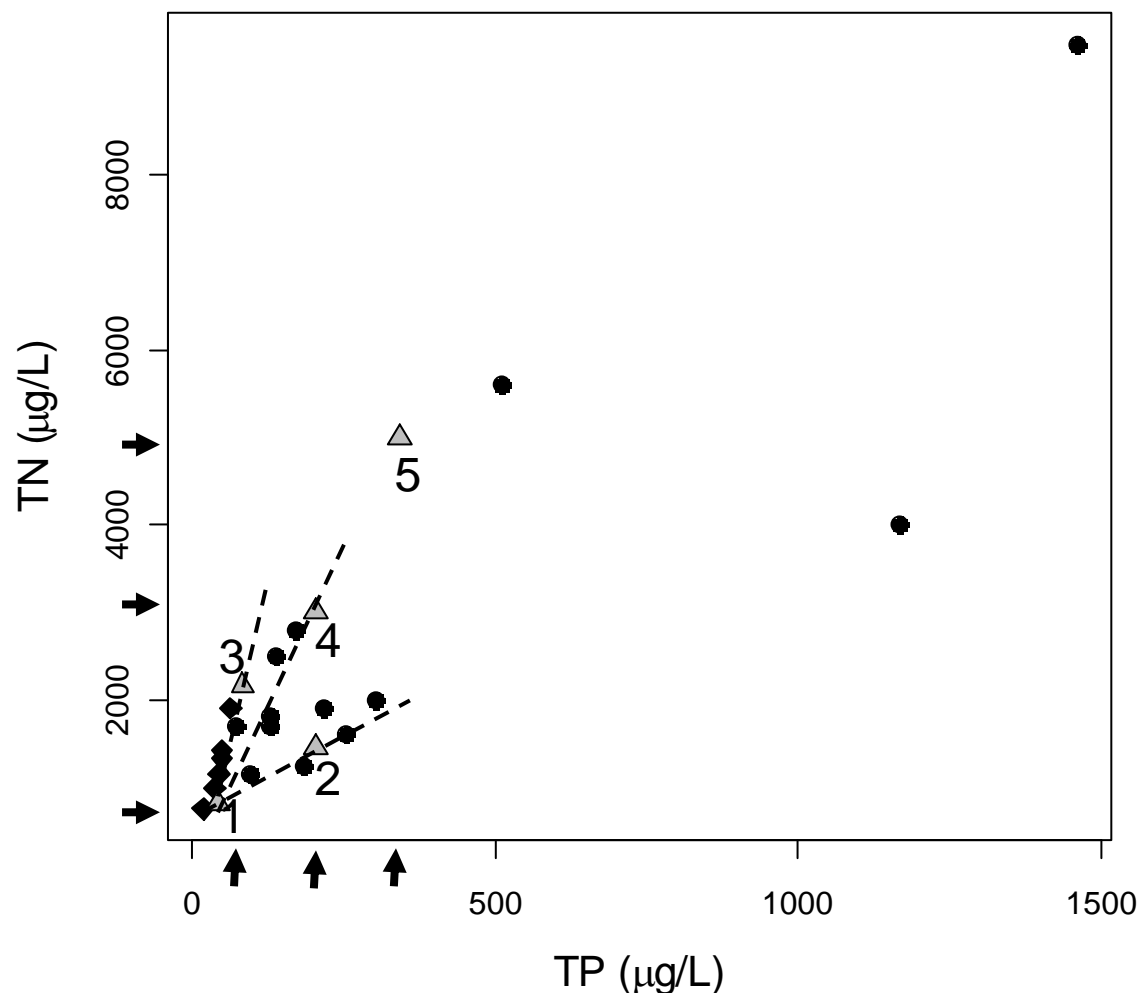


Figure 3.2. Nutrient levels in experimental treatments for mesocosm experiment (grey triangles) and natural pond observations (diamonds = submerged, circles = floating, as in Figure 3.1). Natural ponds were classified by community state using K-means cluster analysis. Treatments are numbered by increasing N levels. Also shown are the levels of N, P, and N:P used in the preplanned contrast for each nutrient variable: the three N levels and P levels are shown with arrows on their respective axes; the slopes of the dotted lines denote the N:P contrast (the longest line shows the target N:P ratio of ~14.6:1, and the shorter lines denote the high and low N:P treatments). See text for details.

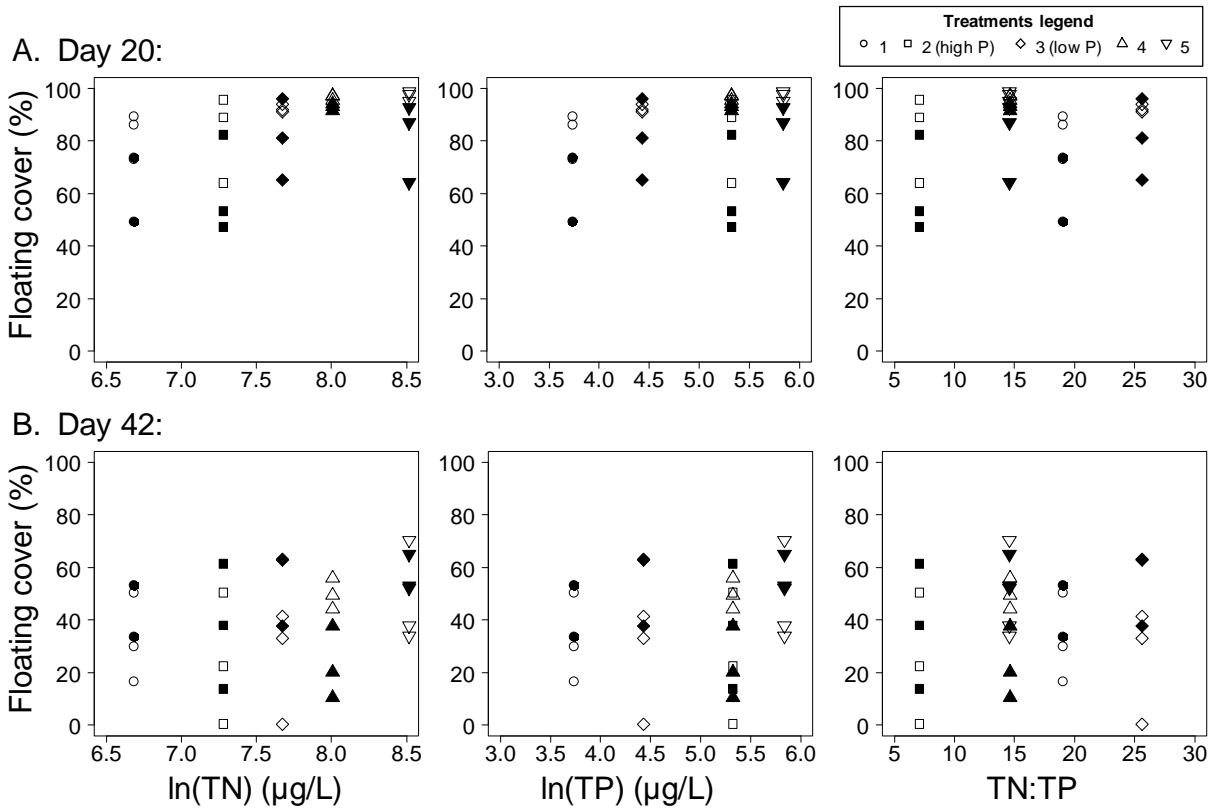


Figure 3.3. Relationship of floating plant abundance (percent cover) with nitrogen (TN), phosphorus (TP), and their ratio (TN:TP) in the nutrient enrichment experiment. Symbols denote nutrient treatment (numbered by increasing target nitrogen levels; see Treatments legend). Shading denotes the experimental pond of each enclosure (open symbols = pond 13, shaded symbols = pond 14). Total N and P levels are ln-transformed.

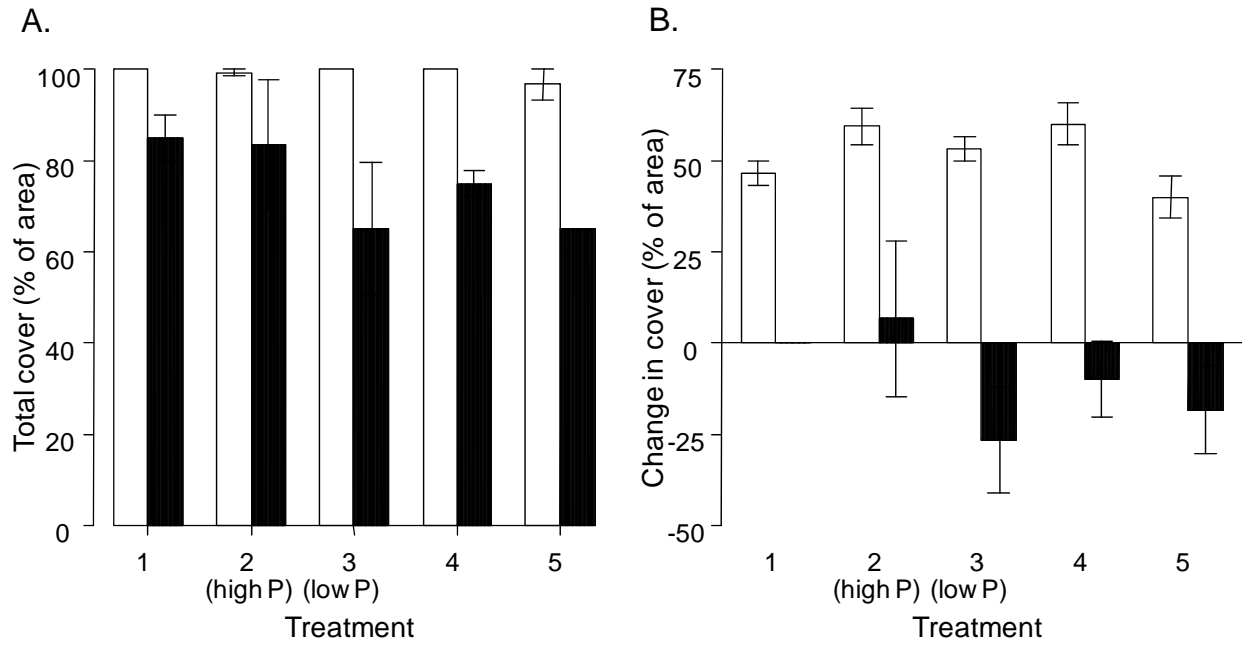


Figure 3.4. Visual estimates of submerged plant cover in the field experiment (mean \pm 1 s.e., $n = 3$, except $n = 2$ in treatment 1 pond 14), expressed as percentages of the basin covered within each enclosure after pond draining. Total cover at the end of the experiment (day 70) and change in cover during the experiment (before the experiment to day 70) are shown versus nutrient addition treatment, separated by pond (open = pond 13, filled = pond 14).

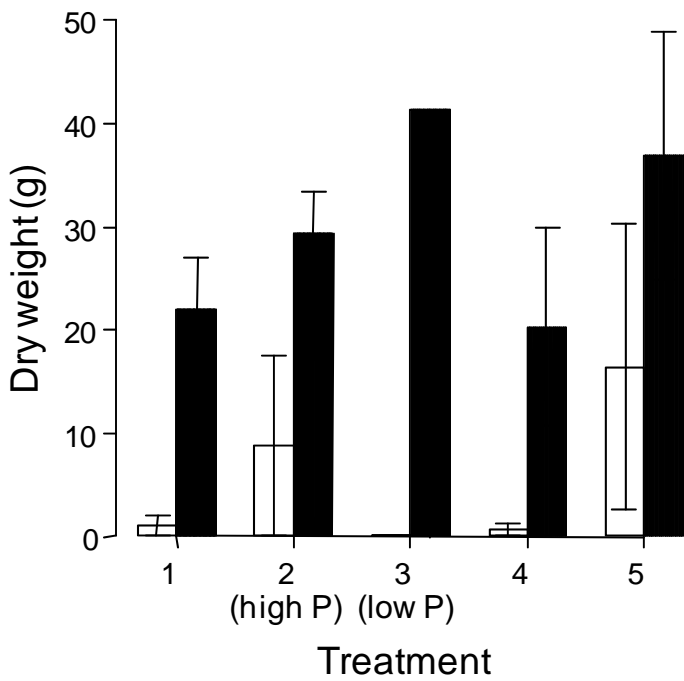


Figure 3.5. Floating algal beds (dried biomass, mean \pm 1 s.e.) that accumulated on the surfaces of the enclosures during the experiment (collected days 66-70) versus nutrient addition treatment. Bars are colored by pond (open = pond 13, filled = pond 14).

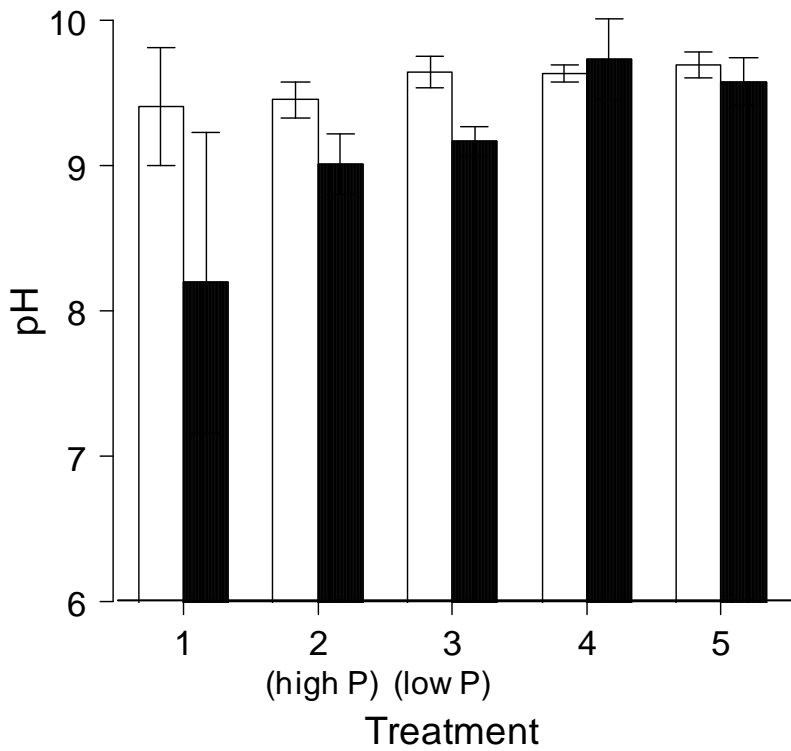


Figure 3.6. pH (mean \pm 1 s.e.) in the experimental enclosures after the end of the experiment (days 61-62). Bars are colored by pond (open = pond 13, filled = pond 14).

CHAPTER 4

THE ROLE OF LIGHT AVAILABILITY IN DETERMINING PRODUCER COMMUNITY STRUCTURE IN TEMPERATE ISOLATED WETLANDS

Abstract:

Nutrient availability has long been proposed to control free-floating plant dominance. In this study, I explored the role of light in mediating this relationship in temperate isolated wetlands. I surveyed producers and abiotic conditions in 35 wetlands in southwest Michigan, USA. In addition, I conducted a factorial experiment in 18-L outdoor mesocosms manipulating nutrient levels, light levels, and initial densities of floating plants. In the observational data, I found that duckweeds could dominate wetlands at nearly the entire range of light conditions, and that at low light levels they dominated systems frequently in intermediate and high nitrogen concentrations. However, emergent and submerged plants could dominate ponds at high light levels, and duckweed abundances had no clear relationship with nutrients under these conditions. In the experiment, I found a light x nutrient interaction at low initial duckweed densities as well, with duckweeds more abundant at low than high light levels. Competition with other plant groups and photoinhibition are discussed as possible mechanisms driving these patterns. In summary, while nutrients are considered a primary driver of free-floating plants, light may play an important role as a secondary driver in determining dominance.

Introduction:

Understanding the conditions under which free-floating plants dominate freshwater systems has attracted both scientific interest and management concern. Floating plants can reach

high abundances in natural and artificial systems, changing phytoplankton composition and excluding submerged macrophytes (Portielje and Roijackers 1995, Scheffer et al. 2003, Morris et al. 2003, O'Farrell et al. 2009). Further, these blooms can alter the structural heterogeneity of habitats, water chemistry, and faunal diversity (Hillman 1961, Meerhoff et al. 2003, Scheffer et al. 2003, Villamagna and Murphy 2010). Several studies of the distributions of floating plants have focused on the role of nutrients, since floating plants often occur in high nitrogen and phosphorus conditions (Sculthorpe 1967, Portielje and Roijackers 1995, Scheffer et al. 2003, Morris et al. 2003). However, the role of other limiting resources, in particular light, in this response has received less attention.

Nutrient and light availability both can affect growth of aquatic producers, including free-floating plants. Since floating plants rely on water column nutrient supplies (Sculthorpe 1967), low nutrient availability may prevent them from growing. This may lead to a curvilinear positive response of floating plants to nutrients. For example, Kufel et al. (2010) found that lesser duckweed *Lemna minor*, a cosmopolitan free-floating plant, exhibited logarithmically increasing growth rates with increased ammonium and soluble reactive phosphorus. Light availability can have a positive effect on floating plant abundances as well. Although they have good access to light in their position at the top of the water column (Sculthorpe 1967), low light has been shown to inhibit growth of some floating plants under some conditions, such as from shading by emergent plants (Hillman 1961, Guy et al. 1990b). However, as light levels get high, growth can level off, since photosynthesis may reach an asymptote due to photoinhibition (Ögren et al. 1984, Guy et al. 1990a,b).

While both nutrients and light levels are important for floating plants individually, interactive effects are possible as well. In other words, nutrients may drive different responses of

floating plants depending on light levels. Predicting whether and how these variables interact to shape floating plant abundances is challenging in natural aquatic communities, where these variables are not always correlated and a variety of other producers (e.g., plant species with submerged and emergent growth forms, phytoplankton, periphyton, metaphyton) compete for the same resources (Colburn 2004, Wetzel 2001).

Understanding the dual roles of light and nutrients may help to explain floating plant dominance in North American isolated wetlands. Free-floating duckweeds (Lemnaceae) occur in and can dominate a variety of wetlands in North America (Jacobs 1947, Keddy 1976, Galatowitsch and van der Valk 1996, Dickinson and Miller 1998, Holland and Burk 1990, Chapter 2). Many features of these wetlands are comparable to the shallow lakes and smaller water bodies in which floating plants and their interactions with other producers have been studied previously (e.g., Hillman 1961, Portielje and Roijackers 1995, Scheffer et al. 2003, O'Farrell et al. 2009). However, one key difference is light availability. Many North American wetlands are heavily forested, with dense tree canopies limiting the amount of light reaching the surfaces of the wetlands. In Michigan wetlands, patterns of dominance by particular plant functional groups (floating, submerged, and emergent growth forms) form recognizable community states (Chapter 2). Further inspection of the roles of nutrients and light in these wetlands may help unravel the causes of dominance of floating plants in these wetlands.

In this study, I explored the role of nutrient and light availability on floating plants in Michigan wetlands. I used observational data to examine how the natural occurrence of floating plants and their competitors varied with light, and whether their associations with nutrient levels differed in high vs. low light conditions. Since the distributions of the other plant groups and light levels were strongly correlated, I then performed a factorial experiment without submerged

plants in outdoor mesocosms. In the experiment, I tested the effects of nutrient additions on the abundances of floating plants at two light levels and at two initial densities of floating plants. These data enabled me to characterize how nutrients and light may jointly shape floating plant abundances in these systems.

Methods:

Field survey methods:

In late July and early August 2006, I measured producer abundances, light levels, and nutrient levels in 35 wetlands. Detailed methods and other analyses for this dataset are presented in Chapter 2. Here, I present details for additional analyses. Wetlands were located in Kalamazoo, Calhoun, and Barry Counties in southwest Michigan, USA.

Plant cover was estimated visually by functional group (Chapter 2). For each pond with > 11% floating cover, I noted if it was a monoculture of *Lemna minor* or *Wolffia* sp. (allowing for small amounts of *Spirodela* sp., *Lemna triscula*, or *Riccia* sp.) rather than a mixture of species. I measured light levels with a light meter (LI-COR LI-185B quantum/radiometer/photometer, Lincoln, NE, USA) held just above the surface near the center of each wetland. These light measurements are expressed as the percentages of light reaching an unforested area, measured in similar cloud cover within a few minutes of the pond measurement (Cáceres et al. 2008). I also measured depth (near the deepest point in each pond) and water color, since both affect light attenuation through the water column. For water color, water was filtered through GF/F glass microfiber filters (Whatman, Maidstone, England) and absorbance was measured at 320 nm. Chlorophyll *a* levels were measured by filtering collected water onto GF/F filters and determining chlorophyll *a* content with cold ethanol extraction and narrow band

fluorimetry (Welschmeyer 1994). Additional water was frozen at -20°C for nutrient analysis (Bachmann and Canfield 1996). Although several nutrients affect producer dynamics in wetlands, total nitrogen levels are focused on here, since nitrogen often is limiting in wetlands (Bedford et al. 1999) and is an important driver of producers in these wetlands (Chapter 3). Total nitrogen (TN) was measured by persulfate digestion and second derivative spectroscopy (Crumpton et al. 1992).

Field survey data analysis:

I evaluated correlations among the abiotic variables (incident light levels, water color, depth, and TN) using Spearman rank correlation tests in R 2.9.2. Spearman tests were more appropriate than Pearson tests, because they detect a variety of monotonic relationships between variables without normality or linearity, assumptions unlikely to hold for these data (Chapter 2, Appendix B). Community structure was determined using cluster analysis (Chapter 2). Connecting the abiotic and producer community data, I examined how plant community structure changed with light levels. I performed a regression tree analysis (CART) to group light levels into categories and to determine if any breakpoints in light levels predicted differences in floating plant cover. The CART algorithm divided the observations into groups by determining breakpoints of light levels (independent variable) that best predicted floating plant cover (dependent variable). I performed this analysis in SYSTAT 10.0 with default settings. I also examined whether there was a shift in floating plant species dominance with light using classification trees (CART) with dominance (*Lemna minor*, *Wolffia* sp., or other dominance) as the dependent variable and incident light levels as the independent variable. I used the subset of ponds dominated by floating plants for this analysis, running the analysis both with and without

ponds of other dominance patterns included (the identified threshold was identical both ways). I present the analysis excluding ponds of other dominance patterns, run using SYSTAT 10.0 with a minimum node size of 3 observations and other settings default.

I evaluated whether the relationship between floating plants and nutrients differed at different light levels. Since the effects of TN have been studied more thoroughly previously using another dataset and the present dataset includes a narrower TN range than the previous one (Chapter 2), I consider TN levels as low, intermediate, or high based on the ranges of values that floating plants never, sometimes, and always dominated in that field dataset (transitions of 1148 and 3111 $\mu\text{g/L}$ TN, Chapter 3). I initially examined the effects of nutrients at different light levels graphically using the light categories identified from CART analysis. For low and medium light levels, I further explored a threshold-like pattern in the response of floating plants to TN with CART in SYSTAT 10.0, using TN as the independent variable and floating cover as the dependent variable for this subset of data (minimum node size of 3 observations, other settings default).

Since floating plants may compete with phytoplankton as well as other true plants in these ponds, I also examined the relationship between floating plant abundances and chlorophyll *a* levels (a proxy of phytoplankton abundance) to determine whether this relationship was positive or negative. A negative relationship may indicate competition with some phytoplankton species, a positive relationship may indicate facilitation with some phytoplankton species (or apparent facilitation, with both functional groups having a common response to the same driver), and no relationship may be expected if the groups coexist with independent dynamics. I performed Pearson correlation tests in R 2.9.2 on the whole dataset. Chlorophyll levels were transformed ($\ln + 1$) prior to analyses to fit normality assumptions better. I also ran partial

correlation tests removing the effect of TN (ln-transformed); results were nearly identical and thus are not presented here.

Mesocosm experiment methods:

To study the effects of nutrients on floating plants at different light levels, I manipulated light (2 levels), nutrients (5 levels), and initial densities (2 levels) with six replicates in a fully factorial design in 18-L mesocosms.

This work was conducted at the Experimental Pond Facility at W.K. Kellogg Biological Station (Michigan State University, Hickory Corners, MI, USA). I created artificial pools in white plastic buckets (~20 L capacity). I regulated temperature in the buckets by positioning them in larger water baths. Since ten buckets fit inside each water bath, the 120 experimental units were divided into blocks in a partially balanced incomplete block design (Kuehl 2000), with one set of 20 treatment combinations contained within two side-by-side pools. Treatments combinations were assigned using an α_n -design (John et al. 2002), an extension of α designs that allows optimal estimation of main and interactive effects. To do this, I used CycDesigN 3.0 demonstration version (Whitaker et al., CSIRO, Canberra), and then I randomized assignments of treatments within blocks to the buckets.

Light was manipulated in two treatments using shade cloth. The high light treatments had only window screen (~1 mm holes) to exclude flying insects. The low light treatment buckets were covered with both the screen and 37% shade cloth. Nutrient supply rates were manipulated with five nutrient addition treatments. Nitrogen and phosphorus both were added as NaNO_3 and NaH_2PO_4 in an N:P ratio of 11.15:1 (the average TN:TP of 31 pond observations with $\geq 70\%$ floating cover across two datasets, Chapters 2 and 3). Nitrogen targets for the initial

addition spanned a wide gradient for the five treatments, with approximate targets of 200 (ambient), 900, 1900, 2650, and 4000 $\mu\text{g/L}$. I typically added nutrients weekly, replenishing for producer uptake at a rate of $\sim 5\%$ loss per day based on previous work in larger mesocosms (M. Leibold, unpublished data), but I sometimes adjusted these replenishments based on actual nutrient levels measured in subsets of the buckets. For the manipulations of initial densities of floating plants, I chose two levels, one low ($\sim 10\%$) and one high ($\sim 75\%$). These cover values are estimates because plants were added by live biomass.

Buckets were cleaned with 10% bleach prior to use. Each bucket was filled with 18 L groundwater and covered with a screen on 22 May 2007. The water was aged three weeks to let dissolved iron precipitate. Also, small amounts of hydrochloric acid were added to all buckets in three additions (last addition on 5 June 2007). This was done to attempt to lower pH, because lemnids grow in acidic conditions (Keddy 1976, Chapter 2). The pH range immediately after this final addition was 4.93 to 6.15 in 17 of the buckets. However, pH levels rose quickly after each acid addition.

Mesocosms were seeded with phytoplankton collected from twelve natural ponds in Barry County on 6 June 2007. I used an 80- μm zooplankton sieve to exclude larger organisms. I added a 100-ml aliquot of phytoplankton to each bucket and began nutrient additions on the same day. Zooplankton were collected from ten of the same ponds on 12 June 2007. Large insects were removed. I added 40-ml aliquots of concentrated zooplankton to each bucket. Snails (collected from artificial habitats around the Experimental Pond Facility) were added to the buckets 27-28 June 2007 after a week in the laboratory for counting and measuring. A mixture of seven *Physa* individuals (0.54 ± 0.03 g total) and two *Helisoma* snails (1.31 ± 0.14 g total) were used (average pooled live masses for 30 of the buckets, mean ± 1 S.D.).

Duckweeds were added 2-3 July 2007 (3 July 2007 is considered day 1 of the experiment). Duckweeds (predominantly *Lemna minor*) were collected from ten ponds in Barry County on 1 July 2007 by skimming a D-frame insect net along the surface of each pond. Duckweeds from each pond were mixed in large tanks, and sieving and manual removal were used to exclude invertebrates and detritus from small batches of plants. Duckweeds were weighed into aliquots after further rinsing, draining, and gentle blotting with paper towels. I added 2.00 ± 0.05 g duckweeds (live mass) for the low density treatment and 20.00 ± 0.05 g duckweeds to the high density treatment. Duckweeds were allowed to spread naturally.

I added aged water as needed (usually weekly) to maintain ~17.5-18.5 L in the buckets. On 7-9 July 2007, I diluted the buckets with extra aged tap water, because I had added 2-3x more nutrient solution than I intended the day before due to miscalculations. To accomplish this, I removed 3 L of the bucket water (saving and adding the zooplankton from it back in), and then added 4 L of aged tap water to bring water levels to 19 L.

I monitored floating taxa abundances using photography. I present cover data from day 16, at the end of the initial growth response of the duckweed. Photographs of the bucket surfaces were taken with a Nikon Coolpix 4500 digital camera (2272x1704 pixel setting; manually adjusted aperture and shutter speed settings). Percent cover of floating taxa was determined from the photographs using image analysis software. The area of interest was designated by coloring everything around the enclosure surface in pure black in Adobe Photoshop 6.0. The contrast levels of the grayscale photos were adjusted to account for differences in exposure, and pixels were classified as floating taxa or open water using threshold values in MATLAB R2009a.

Water (250 ml) was collected from the buckets for chlorophyll *a* determination (analyzed as described above) before the experiment and on day 9. Chlorophyll levels were measured as a proxy of phytoplankton abundance (one competitor with floating taxa in these mesocosms).

Mesocosm experiment data analysis:

I evaluated the effects of the experimental manipulations on floating plant cover using ANOVA. I included the effects of light, nutrient, and density treatments (all fixed factors) and all two-way and three-way interactions. I also included the effects of the two blocking factors (replicate and water bath within replicate) in the original model. However, I dropped them from the model subsequently, because their covariance parameter estimates were not significantly different from zero. These analyses were performed in PROC MIXED in SAS 9.2 with Kenward-Roger degrees of freedom computation. I used the slice function to pull apart the light x nutrient interaction.

I also performed correlation tests between floating plant cover and phytoplankton (chlorophyll *a* levels, $\ln + 1$ transformed) to examine whether there was a positive (facilitation or correlated responses of both groups), neutral (coexistence), or negative (competitive) relationship. I used Pearson partial correlation tests to account for the effect of the nutrient treatments (as \ln -transformed target TN levels). I performed these tests with the data grouped by light and density treatments using `pcor.test` code by S. Yi in R with the variance-covariance matrix method.

Results:

In the observational dataset, floating plants occurred over a wide range of incident light

levels (Figure 4.1A). While the observation with the minimum light value in the dataset was not dominated by duckweed, duckweeds dominated at values as low as 4% of total incident light. In contrast, emergent and submerged vegetation only dominated wetlands with > 73% light levels. Consequently, CART identified two breakpoints that helped to predict floating plant cover with light levels. At low light levels (< 41.2%), only community states dominated by floating plants or few producers in late summer were observed. However, at high light levels (> 78.6%), other plant competitors sometimes dominated or co-dominated wetlands. Ponds with high light levels always had abundant vegetation of at least one plant functional group. While there were no clear patterns between floating cover and water color, deep ponds were more often dominated by duckweed than shallow ponds (Figure 4.1A). There were no notable correlations among the abiotic variables studied (Table 4.1).

While duckweed sometimes dominated ponds under nearly the entire range of light levels, there was a change in species composition with this variable. For the ponds that were monocultures of one species of duckweed, the identity of that species shifted from *Lemna minor* in low light conditions to *Wolffia* sp. in high light conditions (Figure 4.1B). This transition in dominance from *L. minor* to *Wolffia* sp. in floating plant dominated ponds occurred at 61.8% of incident light (CART threshold, PRE = 0.60 with ponds of other dominance patterns removed, n = 8). Examining the relationship between phytoplankton and floating plant abundances for evidence of competition, I found an overall positive relationship between them in the observational dataset (Figure 4.1C; Pearson $r = 0.56$, $t = 3.85$, $df = 33$, $p < 0.001$).

Floating plants had differing relationships with TN in the different light categories identified from CART analysis (Figure 4.2). At low and medium light levels, I found duckweeds to dominate consistently at intermediate nutrient levels. However, three ponds at low light and

high nutrient levels deviated from this pattern, having few late-summer producers. This led to identification of two breakpoints (1131 and 2689 $\mu\text{g/L TN}$, CART with 3 observation minimum node size, PRE = 0.78). Of these three ponds, one was dominated by floating plants in every other year of the five years it has been monitored (Chapters 2 and 3, S. Smith, unpublished data); one pond represented the minimum light level in the dataset (1% of incident light); the third had no apparent characteristics that made it unsuitable for duckweed growth. At high light levels, there was no apparent relationship between floating plants and nutrients, with floating plants potentially dominating (or having intermediate abundances when emergent plants dominated) throughout the entire range of nutrient levels.

In the initial response of floating taxa in the experiment, there was a strong light x nutrient interaction (Figure 4.3, Table 4.2). The interaction was particularly evident visually at low initial duckweed densities (Figure 4.3). In the low light (shaded) treatment, floating plants increased with nutrient supply rates. However, in the high light (unshaded) treatment, floating plants showed a positive response to nutrients at lower nutrient levels, but this positive response lessened at the highest nutrient treatments. This led to a significant difference in floating cover between light levels at the highest nutrient treatment (lsmeans slice for effect of light at nutrient treatment 5, numerator df = 1, denominator df = 100, $F = 16.14$, $p < 0.001$). Two-way interactions with densities were seen as well (Table 4.2, Figure 4.3). Floating plant abundances began to diverge from their initial densities, but the signature of initial density was evident in the floating taxa responses.

While phytoplankton levels initially dropped in some mesocosms with the addition of the duckweed (Figure 4.4), phytoplankton abundances were only sometimes negatively correlated with floating plant abundances in the experiment (Figure 4.5). When grouped by initial density

and light levels, there were positive relationships between chlorophyll levels and floating taxa abundances in the high density treatments (Pearson partial correlation tests; at high light: $r = 0.41$, $n = 30$, $p = 0.02$; at low light: $r = 0.54$, $n = 30$, $p < 0.001$), and negative relationships in the low density treatments (at high light: $r = -0.71$, $n = 30$, $p < 0.001$; at low light: $r = -0.38$, $n = 30$, $p = 0.03$). However, differences by light level in phytoplankton abundances were not strong, thus not explaining the light x nutrient interaction seen in duckweed abundances.

Discussion:

Light levels appeared to play an important role in structuring the producer communities of isolated wetlands in Michigan. From my survey work, I found that ponds with low light availability (i.e. more shaded by tree canopies) either were dominated by floating plants or no plants, and they were almost always dominated by floating plants at intermediate nutrient levels. However, wetlands with high light availability often had dominance of other plant competitors. Studying this interaction in the absence of other plant competitors in the mesocosm experiment, I also observed that light levels affected the response of floating plants to nutrients. Thus, both variables may interact to shape floating plant abundances.

Competitive interactions among plant taxa may drive this light x nutrient interaction. One way is via niche differences, since floating plants dominated at lower light levels than submerged and emergent plants. Despite past work showing that duckweeds can exhibit lower growth with lower light availability (Hillman 1961), I found that floating plants were able to dominate at almost the entire range of light availability in these wetlands. Explanations for floating plants persisting even in low light include that lemniids can exhibit heterotrophy (Hillman 1961) and/or that they carry out most of their growth early in a season before leaf-out

of tree canopies (S. Hamilton, pers. comm.). Also, it is possible that the plants still photosynthesize at low light levels, since optimal light levels for some floating plants are relatively low (Hillman 1961, Ögren et al. 1984, Guy et al. 1990a,b).

Some previous work in shallow lakes has focused on a more complex pattern resulting from the interaction between submerged and floating plants. At high light levels, asymmetric competition between floating and submerged plants for nutrients and light can lead to an S-shaped nonlinear response to nutrients referred to as a hysteresis (Scheffer et al. 2003). However, when lake depth and/or turbidity are high, light availability in the water column may be reduced sufficiently to exclude underwater competitors such as submerged plants. These low light levels would lead to a simpler response of floating plant to nutrients, such as a logarithmic response (Kufel et al. 2010). These predictions mostly matched my field patterns, with a threshold-like response of floating plants to nutrients at lower light levels but possible dominance of submerged and emergent plants at high light levels. While the added light reduction from tree canopies distinguishes these Michigan wetlands from the shallow lakes in which this hypothesis has previously been examined, these mechanisms could translate directly. Resulting patterns in forested wetlands may even be more exaggerated by the reduction of light entering the water column before attenuation, since this further lowers the amount of light reaching producers near the sediment. The observation that deeper ponds were more likely to be duckweed-dominated fit well with this hypothesis as well, since attenuation increases with depth (Wetzel 2001).

While competition with other plants may explain the light x nutrient interaction in the field data, I also observed this interaction in the absence of other plants in the mesocosm experiment. This observation suggests that competition with plants is not the only possible

mechanism producing these patterns. One alternative is competition with phytoplankton, but much of the data presented here did not support this explanation. These data better fit past research suggesting a positive association between some phytoplankton and floating plants, since some phytoplankton species coexist with floating plants (e.g., with body shapes optimized for light absorption) (O'Farrell et al. 2007, O'Farrell et al. 2009). Positive correlations between floating plants and phytoplankton also may have resulted from phytoplankton increasing their per cell chlorophyll contents rather than exhibiting higher abundances (Geider et al. 1996). Since only chlorophyll *a* levels are available in this study, I cannot separate these two possibilities, and they are not mutually exclusive.

Another explanation for the different responses of floating plants to nutrients at different light levels is based on their physiology. Studies have shown *L. minor* and *L. gibba* to exhibit poor growth at high light levels due to photoinhibition (excess vibrations of photosynthetic machinery inhibiting photosynthesis) and concurrent higher temperatures (Hillman 1961, Ögren et al. 1984, Guy et al. 1990a,b). Optimal photosynthetic rates may occur at relatively low light levels (e.g., *Lemna gibba* exhibited saturation at $\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux densities: Guy et al. 1990a), leading to reduced responses to nutrients under high light conditions. Other observations were in line with this hypothesis, such as the dominance of *Wolffia* sp. instead of *L. minor* at high light levels in the observational data, and the lower abundances of duckweeds at high light levels in low density, high nutrient treatments in the experiment (which were mostly *L. minor*).

Overall, this work supports the hypothesis that light may act as a secondary driver changing the response of floating taxa to nutrient availability under some conditions. Discussion of the role of secondary drivers changing the effects of primary drivers is receiving increasing

attention with anthropogenic forcing (Chapin et al. 2006, Groffman et al. 2006). While the relative importance of different mechanisms in driving these patterns is unclear, this study offers empirical evidence for interactions among drivers in wetland producer communities. Further experimental work to explore these interactions among drivers may be fruitful.

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Figures and Tables:

Table 4.1. Correlation matrix of abiotic variables measured in southwest Michigan wetlands (n = 35 wetlands, except n = 34 for water color). Shown are Spearman rank correlation coefficients of untransformed data (with approximate p-values for tests of significance from 0 in parentheses).

	Light	Depth	Water color
Depth	0.03 (p = 0.89)		
Water color	-0.31 (p = 0.08)	-0.21 (p = 0.24)	
TN	0.11 (p = 0.52)	-0.08 (p = 0.64)	0.27 (p = 0.12)

Table 4.2. Responses of floating taxa to treatments at day 16 of the outdoor mesocosm experiment using ANOVA. Random blocking effects were excluded here, because they were not significantly different from zero. Residual effects were 55.17 ± 7.80 (mean \pm s.e.). (Abbreviations used: Num DF = numerator degrees of freedom, Den DF = denominator degrees of freedom, F = F-statistic, p = p-value.)

Effect	Num DF	Den DF	F	p
light	1	100	3.83	0.05
density	1	100	1509.03	< 0.0001
nutrient	4	100	14.32	< 0.0001
light x density	1	100	10.63	0.002
light x nutrient	4	100	3.81	0.01
density x nutrient	4	100	2.79	0.03
light x density x nutrient	4	100	1.02	0.40

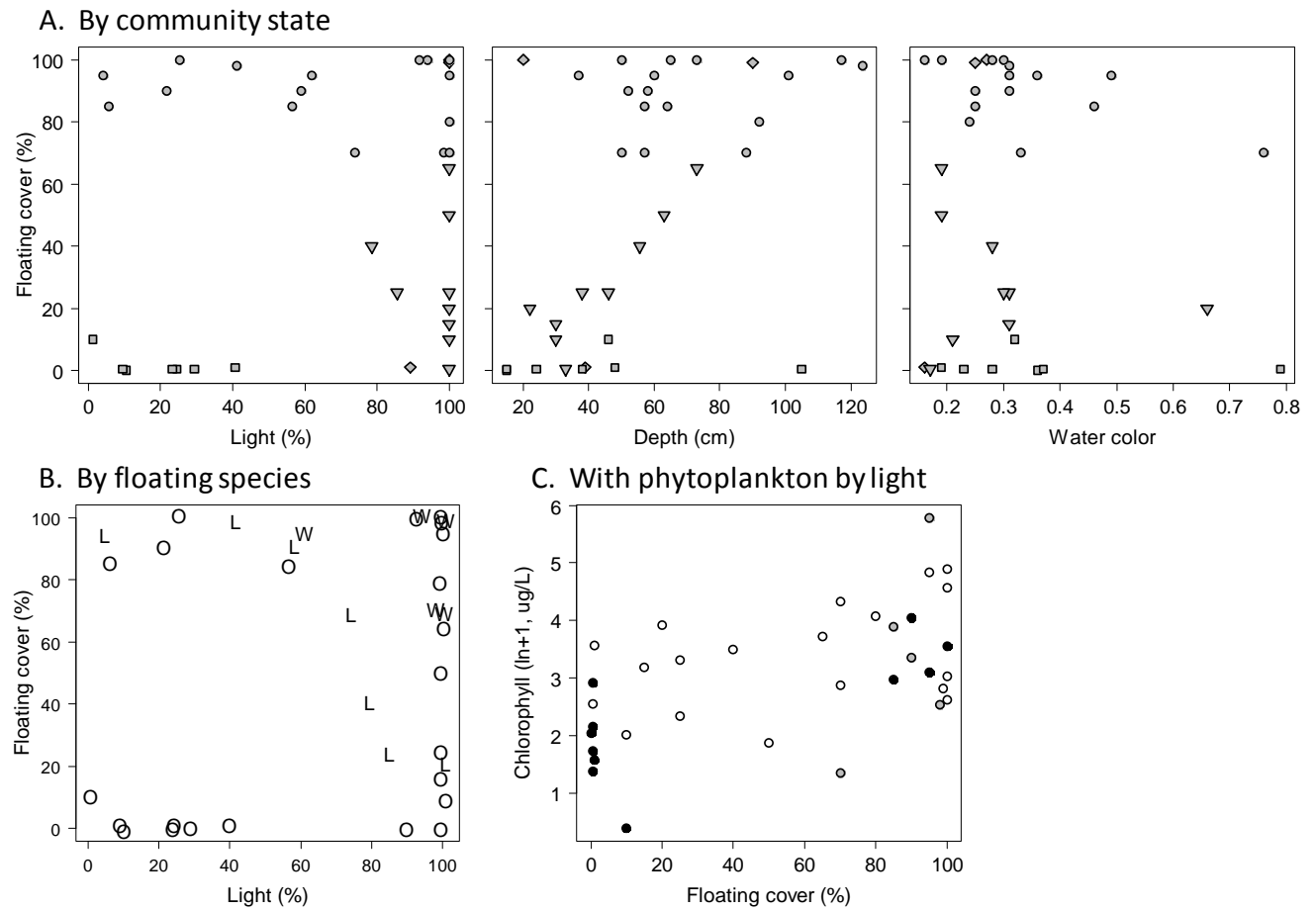


Figure 4.1. Survey data showing the relationships between producer abundances and light in 35 wetlands in southwest Michigan (late summer 2006). (A) Relationship between floating cover (% of pond covered) vs. light variables, sorted by community state: dominance of floating (circles), emergent (triangles), submerged (diamonds), or few (squares) plants (see Chapter 2 for details). Light variables are light penetration to the pond surfaces (% of incident light), depths (approximate maxima), and water color (absorbance at 320 nm of filtered water). (B) Wetlands plotted as in (A), but grouped by duckweed species dominance: L = *Lemna minor* dominance, W = *Wolffia* sp. dominance, O = other dominance (mixed polycultures, only *Spirodela* sp., or no duckweed). (C) Relationship between phytoplankton abundance (chlorophyll *a* levels, shown as ln+1 transformation of data in $\mu\text{g/L}$) and floating plant abundance. Points are colored by light levels (black = low light, grey = medium light, white = high light, based on CART thresholds).

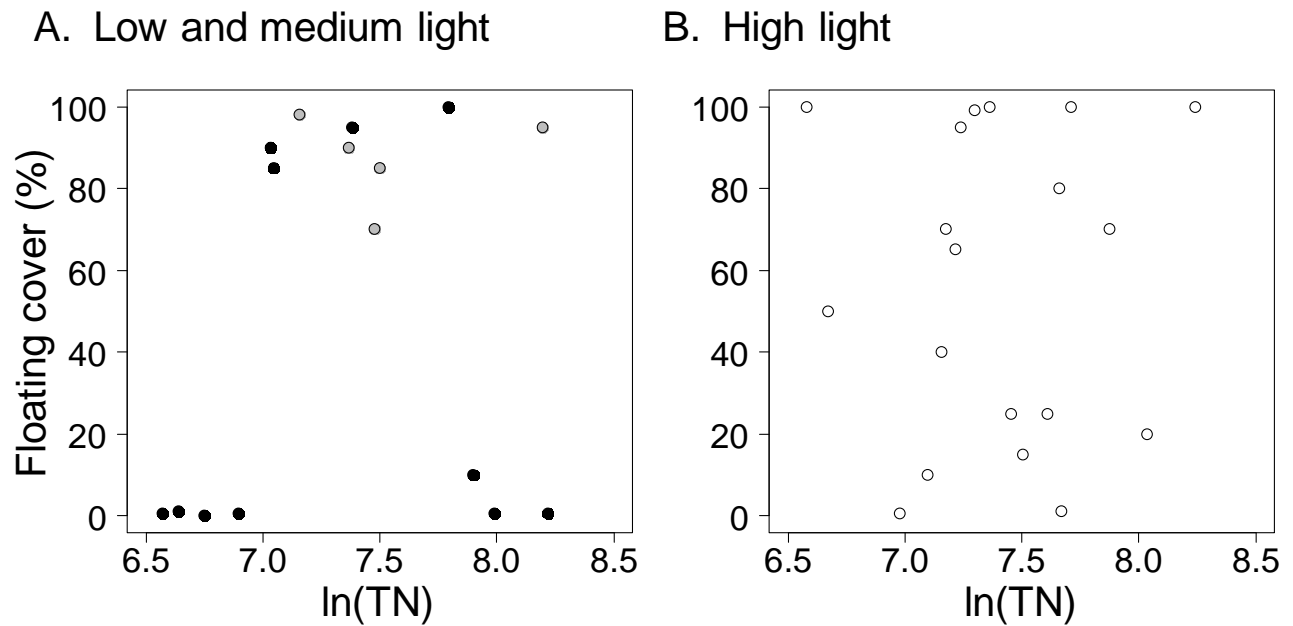
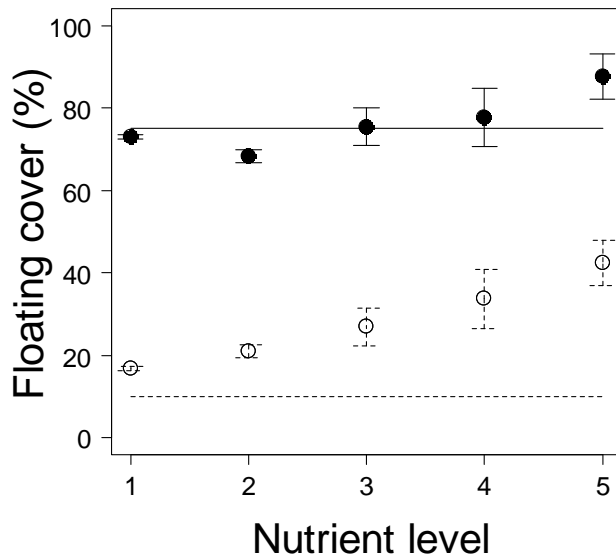


Figure 4.2. Relationship between floating plant cover and total nitrogen levels in 35 wetlands in southwest Michigan, separated by light levels. Ponds with low (black) and medium (grey) light levels both are plotted in (A), since both are characterized by the absence of submerged and emergent vegetation. Ponds with high (white) light levels are plotted in (B). See text for further details.

A. Low light



B. High light

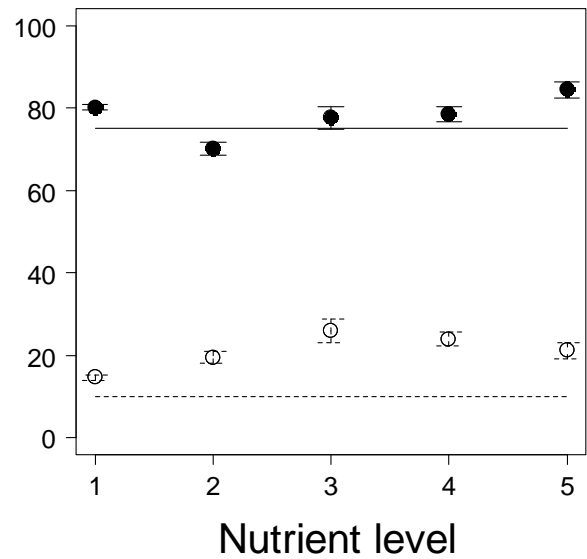


Figure 4.3. Floating plant abundances in the mesocosm experiment manipulating nutrients, light levels, and initial densities on day 16. Floating cover is plotted versus nutrient addition treatment. Dots represent percent cover (mean \pm 1 s.e., solid for high initial density treatment, open and dashed for low initial density treatment). Horizontal lines represent approximate initial densities at the beginning of the experiment for each density treatment. Separate panels are used for the two shading treatments.

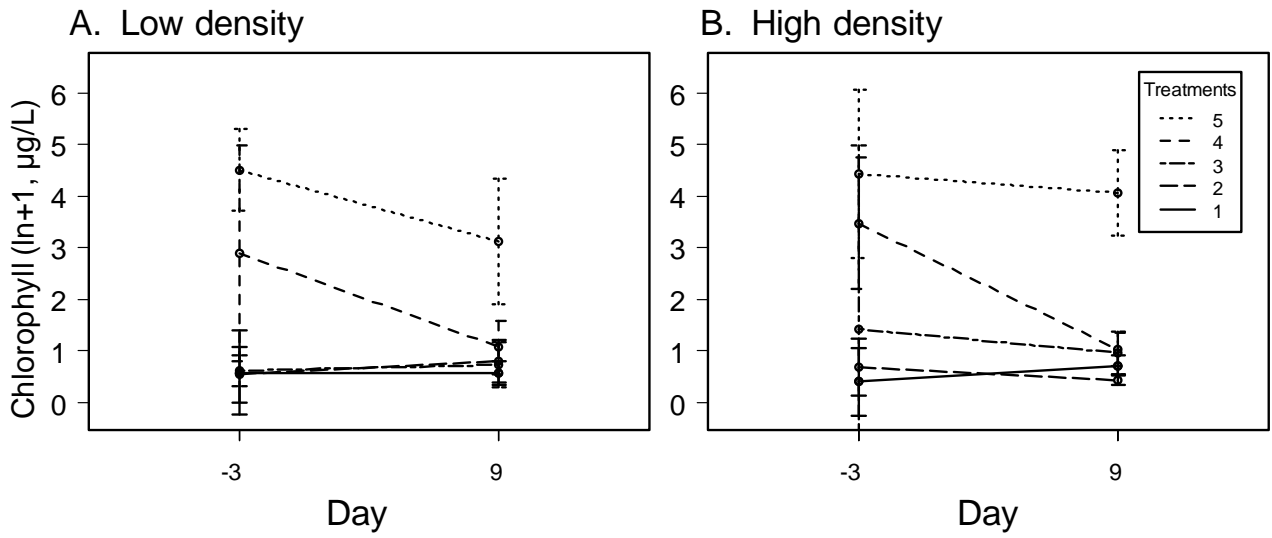


Figure 4.4. Time trajectories of chlorophyll *a* levels (an index of phytoplankton abundance; $\ln + 1$ transformed; mean \pm 95% confidence interval around the means) in the mesocosm experiment. These graphs show both light treatments pooled, since differences by light level were not strong.

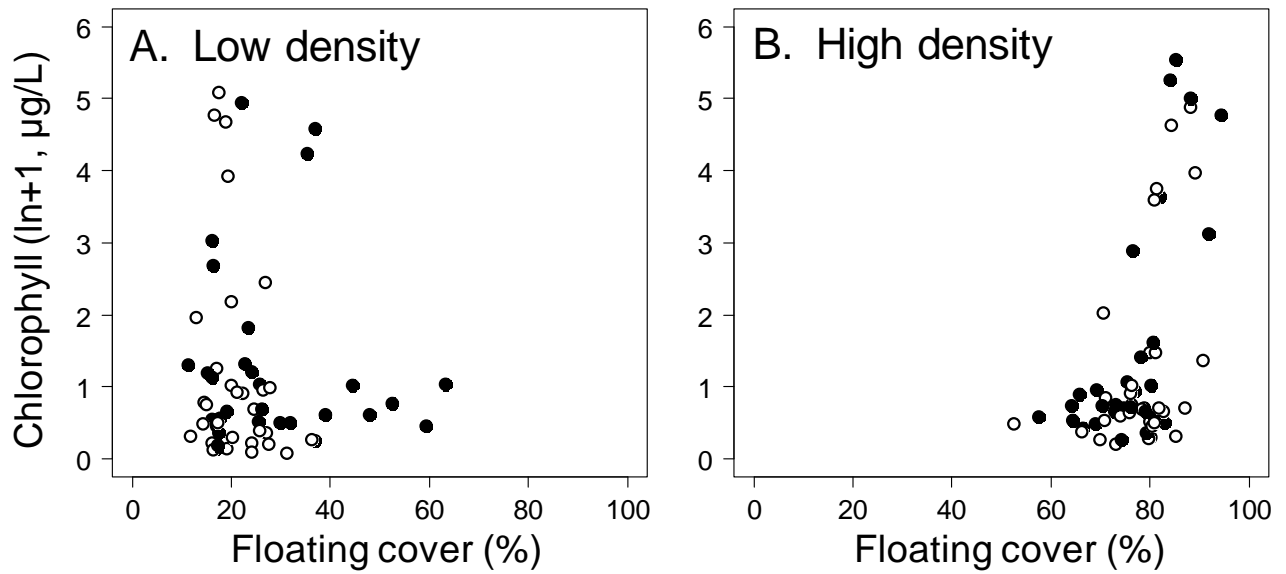


Figure 4.5. Relationship between phytoplankton (chlorophyll *a* levels, $\ln+1$ transformed data in $\mu\text{g/L}$) at day 9 versus floating plant cover at day 16 in the mesocosm experiment, separated into panels by density treatment. Filled symbols are low light treatment; open symbols are high light treatment.

APPENDIX A

ADDITIONAL METHODS AND RESULTS CHARACTERIZING PLANT COMMUNITY COMPOSITION IN MICHIGAN WETLANDS

Methods:

I characterized variability in plant community composition within a season from surveys approximately every other week in April-September 2005. As this was the first year of my study, my data collection methods differed from those described in the main text and evolved over the season. To convert these different data sources into a common form for analysis, I scored plant abundance by cover classes (1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-95%, 6 = 96-100%, as in Daubenmire 1959). I pooled emergent plant cover with submerged and/or floating plants during original data collection. For this analysis, rather than separately quantifying emergent plant cover, I maintained my original data collection methods and used only two groups: surface plant cover (floating and emergent vegetation above the water surface) and rooted plant cover (submerged and emergent vegetation). For surface plant cover classes, I used visual estimates of cover recorded in the field (when available) or based on voucher photographs. (I collected photographic vouchers of plant cover during each observation every year by photographing both the whole wetland from its edge and smaller portions of the surface in five 0.5 x 0.5 m floating quadrats, placed as described in the main text, using a digital camera (Nikon Coolpix 4500; 2272x1704 pixel setting; manually adjusted aperture and shutter speed settings).) Cover classes of rooted plant cover is an estimate of occupancy within sinking quadrats as above, but original data were in the form of quadrat presence/absence, counts of the number of stems, and/or aboveground biomass measurements (oven-dried and weighed) along

with field notes. To convert these 2005 data to the same format as the other years for the annual time series, I used the voucher photographs, field notes, and species level data to separate emergent vegetation from the other plant groups.

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Figures and Tables:

Appendix Table A.1. Summary statistics for univariate distributions of producer functional groups in 35 temporary and semipermanent wetlands in Michigan, USA. Plants are expressed as percent cover (0-100%).

	Min	Max	Median	Mean \pm 1 s.e	Kurtosis (G2) \pm 1 s.e. ¹	Skew (g1) ²	Significance of g1	KS from uniform ³	Significance of KS	Dip (D) \pm 1 s.e. ⁴	Significance of D
Floating plants (%)	0	100	70	53.9 \pm 6.9	-1.76 \pm 0.78	-0.204	n.s.	0.25	0.025	0.113 \pm 0.024	~p < 0.002
Submerged plants (%)	0	96	0	9.7 \pm 4.2	6.55 \pm 0.78	2.623	P < 0.002	0.799	< 0.001	0.028 \pm 0.014	n.s.
Emergent plants (%)	0	100	0	25.0 \pm 6.3	-0.46 \pm 0.78	1.086	0.02 < p < 0.01	0.557	< 0.001	0.067 \pm 0.022	n.s.
Chlorophyll <i>a</i> (μ g/L, not transformed)	0.483	325.5	19.779	39.5 \pm 10.1	15.82 \pm 0.78	3.464	P < 0.002	0.679	< 0.001	0.032 \pm 0.011	n.s.

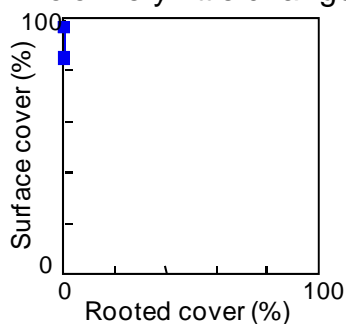
1 Kurtosis is type "G2" in SYSTAT.

2 Skewness is the older definition (type 1 in e1071 package of R), with significance based on Zar (1999). Examining SYSTAT's G1/SES, as described in the SYSTAT 10.0 help files, gave comparable results.

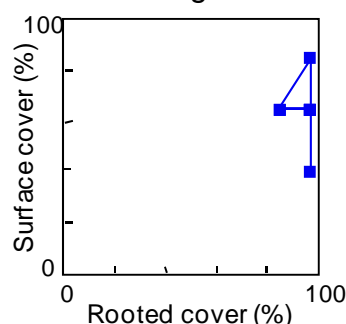
3 Kolmogorov-Smirnov statistic in SYSTAT is based on the maximum difference between the sample distribution and a uniform distribution.

4 Hartigan and Hartigan's (1985) dip test. Standard error computed by bootstrapping. Significance is approximate (using n = 30) from table in diptest package in R.

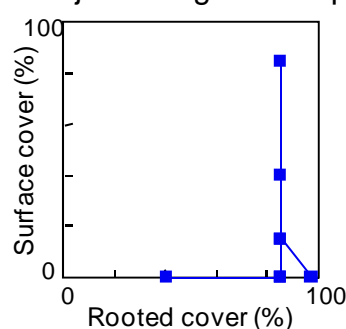
A. No or very little change in abundance



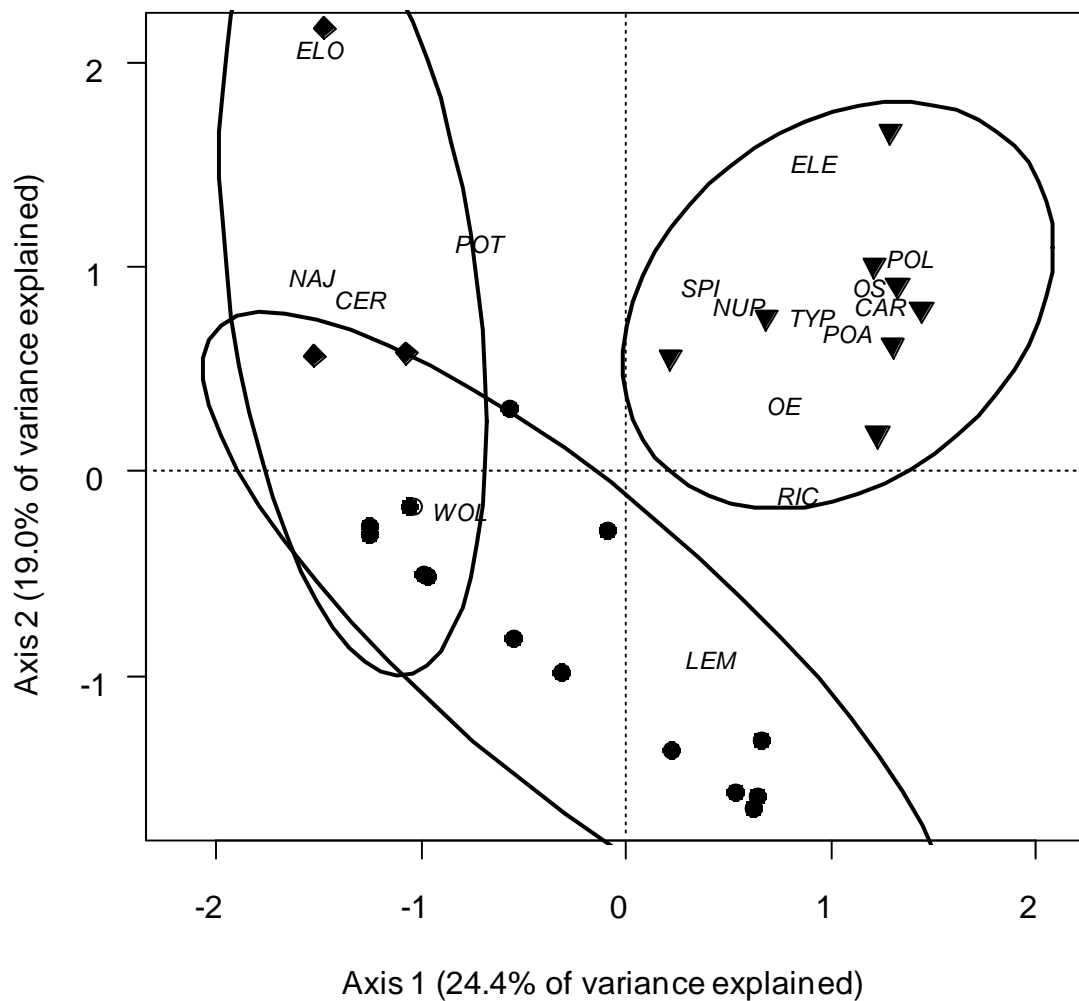
B. Minor change in abundance



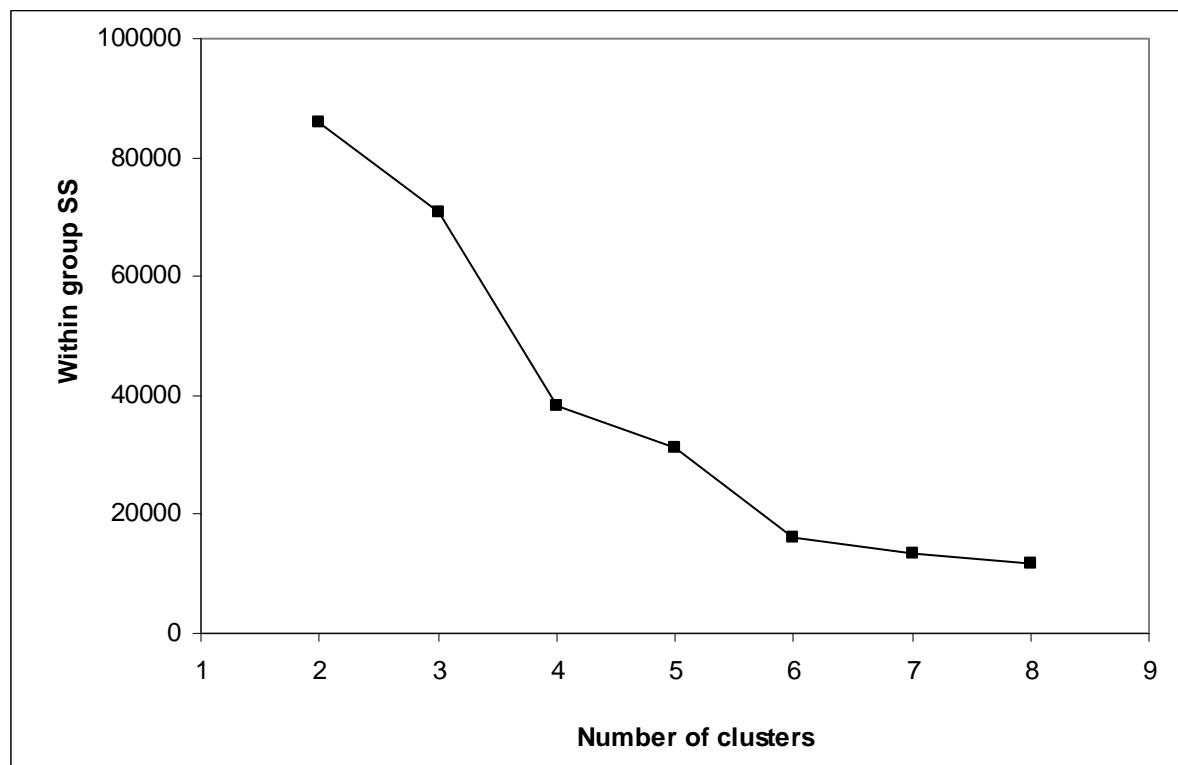
C. Major change in composition



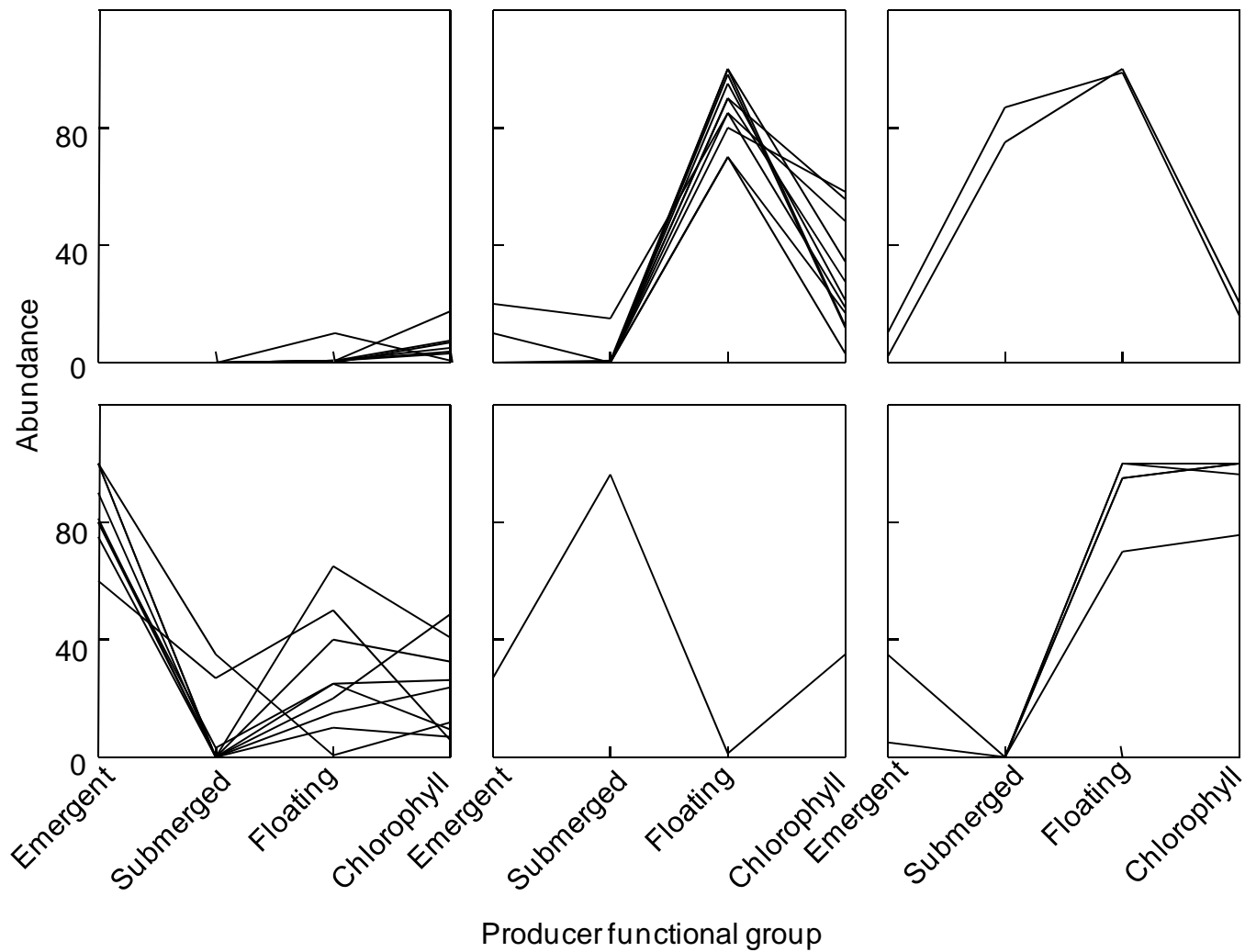
Appendix Figure A.1. Examples of the seasonal variation exhibited by producers of 21 temporary and semipermanent wetlands, southwest Michigan, USA. Data were collected approximately every other week (April-September 2005). Points represent the middle of each cover class range, and lines connect the points in temporal order. Of the 21 wetlands, most exhibited very little change in abundance or composition of plants throughout the season (A) or minor changes in vegetative cover without major changes in composition (B). However, one site (Running Plots Pond at Kellogg Bird Sanctuary, Kalamazoo County, MI) exhibited a strong community shift towards the end of the season (C). Shown here is plant cover above the surface (floating and emergent) vs. plant cover that was rooted (submerged and emergent).



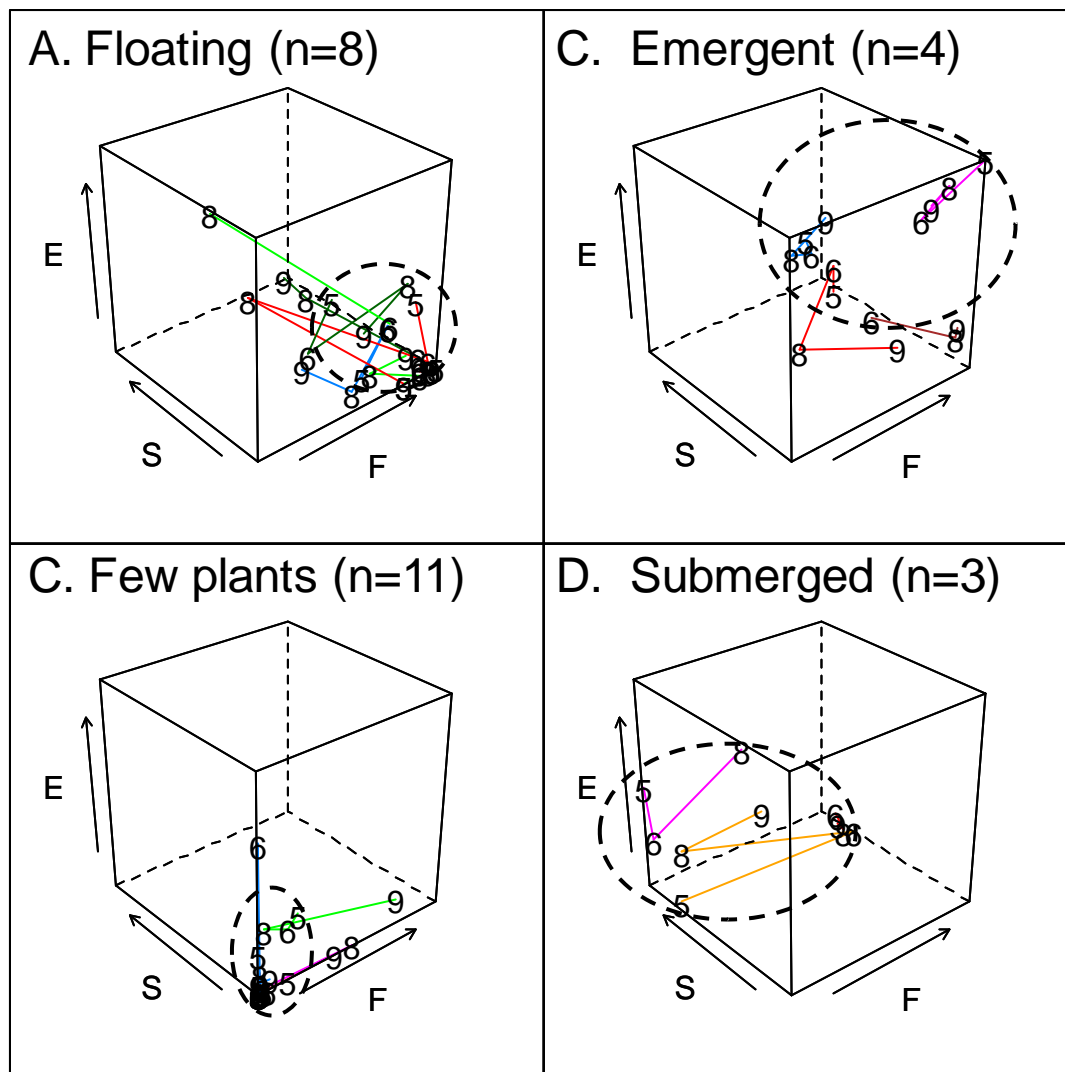
Appendix Figure A.2. Correspondence analysis (unconstrained ordination) of individual plant taxa in temporary and semipermanent wetlands in southwest Michigan, USA. Sites with plant cover >10% and complete water chemistry data were used (as in CCA). One additional site was removed prior to this analysis, because its high abundance of rare species caused it to be overweighted in the analysis. The eigenvalues of the first two axes were 0.695 and 0.541. Site symbols represent community type, as identified by K-means cluster analysis (defined by plant functional group dominance: circles = floating, triangles = emergent, diamonds = submerged). Ellipses are 95% confidence limits for each community state based on the S.D. of the site scores. Taxon abbreviations (genera unless otherwise stated): CAR = sedges (family Cyperaceae--often *Carex*), CER = *Ceratophyllum*, ELE = *Eleocharis*, ELO = *Elodea*, LEM = *Lemna*, NAJ = *Najas*, NUP = *Nuphar*, OE = other less common emergent genera, OS = other less common submerged genera, POA = grasses (family Poaceae), POL = *Polygonum*, POT = *Potamogeton*, RIC = *Riccia*, SPI = *Spirodela*, TYP = *Typha*, WOL = *Wolffia*.



Appendix Figure A.3. Modified Scree plot used to choose the number of groups to include in the K-means cluster analysis shown in Figures 2.3 and A.4. Here, four and six clusters best reduce the within group sums of squares as compared to addition of one more cluster. While these results vary slightly depending on the distance metrics and variables chosen (e.g., how chlorophyll *a* is manipulated to convert it to the same measurement scale as the plants), all variations produced four to seven fairly uniform groups best explaining the variation within the dataset.



Appendix Figure A.4. Cluster analysis results using six clusters instead of four. See Chapter 2 and Figure 2.3 for details. As compared to the four cluster solution presented in Figure 2.3, the wetlands codominated by two producer types (submerged with high floating plant abundance, and floating with high phytoplankton abundance) move into separate groups here.



Appendix Figure A.5. Annual variability in community structure in temporary and semipermanent wetlands in Michigan, USA. This shows the magnitudes and directions of shifts in community composition from year to year. Sites are divided into four panels based on the community states of each site in the first year of sampling: dominance of floating plants (A), emergent plants (B), few producers (C), or submerged plants (D). Each axis represents visual estimates (0-100%) of abundances of one plant functional group (F = floating, S = submerged, and E = emergent plants). Data points are visual estimates from 2005, 2006, 2008, and 2009 (with numbers denoting the last digit of the year), and colored lines connect the observations for each site by year sequentially. (Some sites have a subset of these years due to missing data.) Ellipses were hand drawn to represent the approximate range of each community state by encompassing every initial data point. Thus, lines connecting to points outside of the ellipses can be interpreted large shifts in community state.

APPENDIX B

ADDITIONAL METHODS AND RESULTS CHARACTERIZING POSSIBLE DRIVERS OF MICHIGAN WETLAND PLANT COMMUNITIES

Methods:

I measured maximum depth with a meter stick at the deepest part of the pond. Conductivity (Orion Model 126, Thermo Scientific, Waltham, MA, USA), pH (Oakton Instruments pHTestr 3+, Vernon Hills, IL, USA), and dissolved oxygen (YSI Model 55, Yellow Springs, OH, USA) were measured with separate probes. I measured light levels with a light meter (LI-COR LI-185B quantum/radiometer/photometer, Lincoln, NE, USA) held just above the surface near the center of each wetland; these data are expressed as the percentages of light reaching an unforested area (Cáceres et al. 2008). Water samples to measure nutrients were transported to the laboratory on ice and frozen at -20°C until analysis (Bachmann and Canfield 1996). Total nitrogen was measured by persulfate digestion and second derivative spectroscopy using a Shimadzu UV-2401 PC spectrophotometer (Crumpton et al. 1992). Total phosphorus was measured with this spectrophotometer using the ascorbic acid method after persulfate digestion (Eaton et al. 2005). Finally, for permanence, I scored sites based on whether they dried at the end of the summer in 2005 (a moderately wet year) or not. This is similar to Chase's (2003) distinction of whether ponds dried most years (temporary) or rarely-never (semipermanent or permanent). For wetlands that I did not sample in 2005, I extrapolated this permanence score only for sites that I had surveyed extensively in other years.

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Figures and Tables:

Appendix Table B.1. Discriminant function analysis results for 34 wetlands sampled in July-August 2006. Community state was predicted based on 8 environmental variables (weighted in the canonical functions as listed in (A)). Panel B shows the percentage of observations the linear statistical model classified correctly (both by actual community state and overall). Permanence is not included in this analysis. (Abbreviations used: TN = total nitrogen, TP = total phosphorus.)

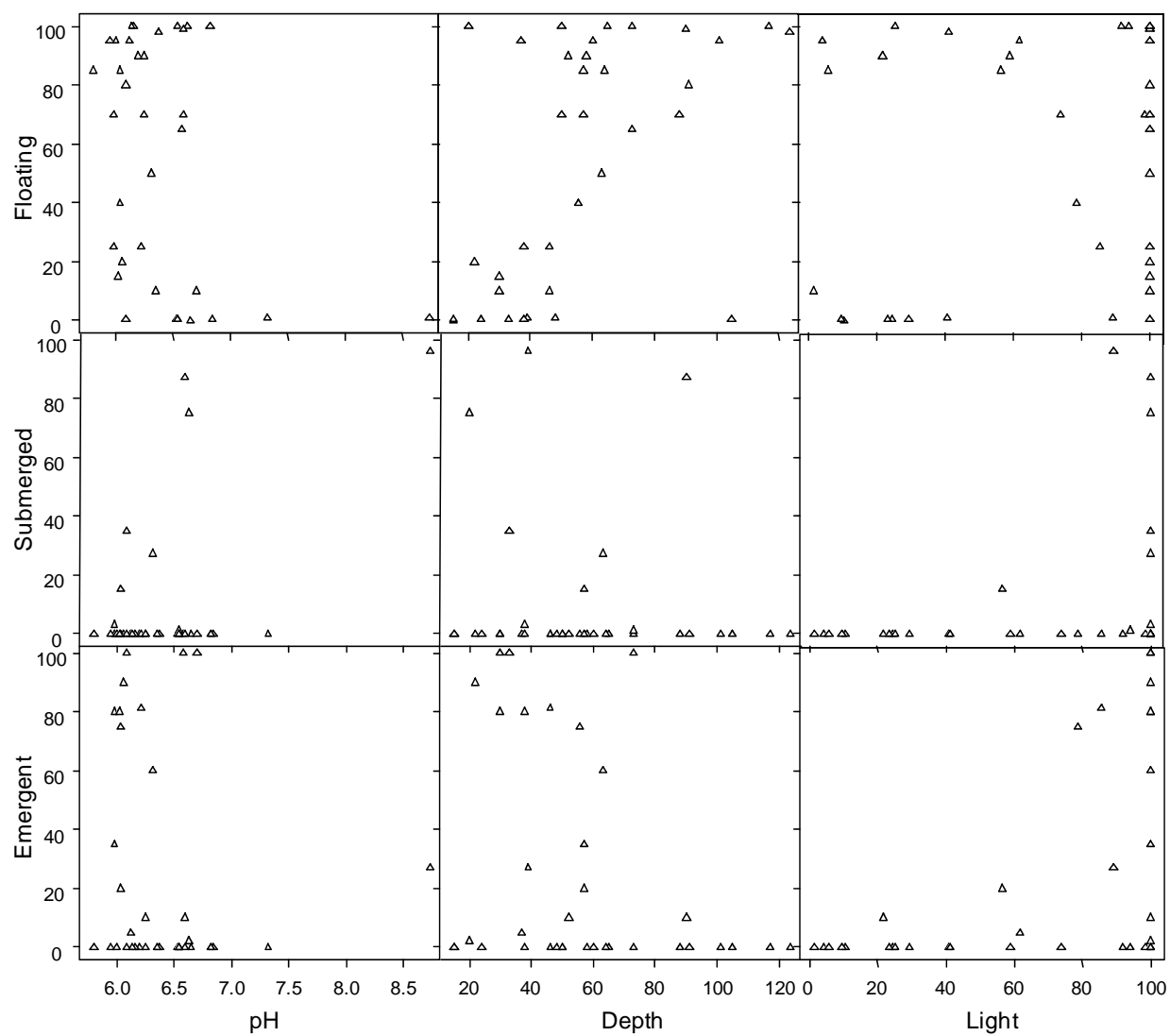
A. Canonical discriminant functions standardized by within variances

Variable	Axis 1	Axis 2	Axis 3
Depth	0.142	0.901	0.719
Dissolved oxygen	0.087	0.499	0.467
Conductivity	-0.390	0.334	-0.156
pH	-0.526	-0.839	0.532
Light	0.797	-0.519	-0.082
TN:TP	0.028	0.735	0.054
ln(TN)	-0.154	-0.241	0.577
ln(TP)	-0.209	0.245	-0.374

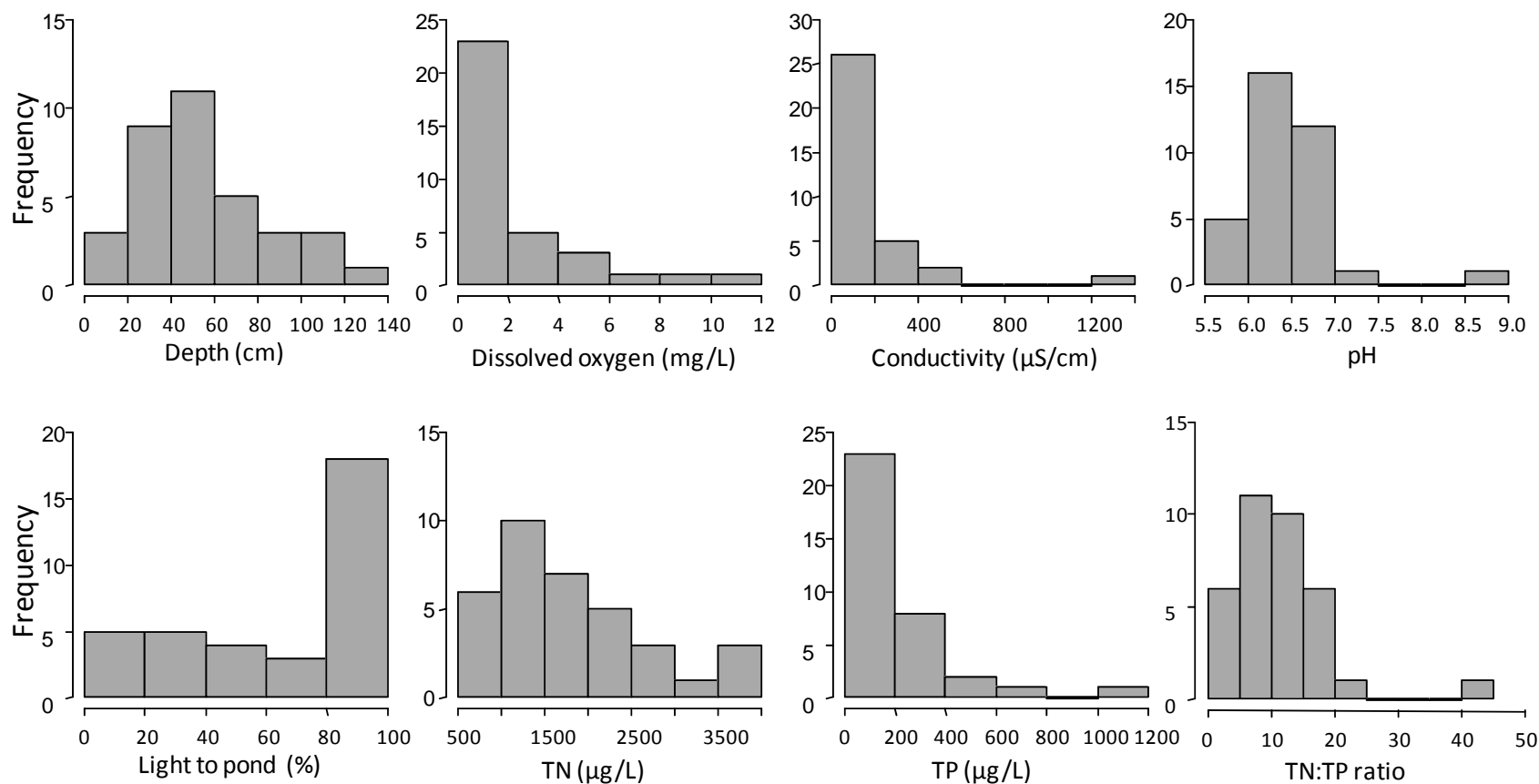
Table B.1 (cont.)

B. Jackknifed classification matrix

<i>Actual community state</i>	<i>Predicted community state</i>				% correct
	Emergent	Floating	Few	Submerged	
Emergent	8	1	0	0	89
Floating	4	8	3	0	53
Few producers	0	1	5	1	71
Submerged	2	0	1	0	0
<i>Totals for all wetlands</i>	<i>14</i>	<i>10</i>	<i>9</i>	<i>1</i>	<i>62</i>



Appendix Figure B.1. Scatterplot matrix of plant functional group abundances versus three abiotic variables for 35 wetlands in southwest Michigan, USA. These abiotic variables (pH, depth, and light) are shown because they were weighted heavily in the three multivariate analyses of possible drivers.



Appendix Figure B.2. Univariate histograms of environmental variables that may represent drivers of producer communities in temporary and semipermanent wetlands in southwest Michigan, USA. Note that scale of y-axis differs among plots. (Variable abbreviations used: TN = total nitrogen, TP = total phosphorus.)